The Biotechnology of Ethanol

Classical and Future Applications

Edited by M. Roehr

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Preface

After two centuries of almost absolute belief in technical and economic progress, human society is in a period of reconsideration and elaboration of new strategies for the ongoing new century. Progress of our civilization with an explosive rise in world population has led to an enormously increased consumption of resources and to an equal threat to the environment. Coping with these problems requires all intellectual abilities of our society. In this endeavor, biotechnology is considered to play a significant role. Notably the question of responsible use of resources for food, energy, and alternative products and production processes has created various reasonable solutions following the crisis in the early 1970s – new routes, but also rediscoveries of others which have been developed under different conditions in the past.

One of the examples discussed as possible alternative and investigated during the last few decades is the production of ethanol from various feedstocks.

The objective of the present book is to provide a concise overview on the state-of-the-art of the manufacture of this valuable commodity which can be utilized in various fields of applications. Biotechnologists as well as other people engaged in considering alternative ways of the sustainable use of renewable resources will find information and useful examples.

In Part I, written by Thomas Senn and Hans Joachim Pieper, University of Hohenheim, Germany, displays the present knowledge of modern distillery technology as carried out in most European countries, using mainly common starch-containing feedstocks. Needless to say that the latest developments of raw materials processing and fermentation technology, particularly considering the difficult energy economics, are covered.

Part II, written by Naim Kosaric, University of Western Ontario, London, Canada, and Fazilet Vardar-Sukan, Ege University of Izmir, Turkey, a wealth of information regarding the use and processing of mainly unconventional raw materials is provided, and special applications are treated, particularly emphasizing the economic and ecological constraints. Case studies and various calculation examples are presented to enable the reader to become familiar with the various considerations to be taken into account, if alcohol be produced for different applications such as fuel or as a commodity in the chemical industry. Special attention is directed to the case of motor fuel additions and the respective implications.

Inevitably, there might be some overlap between the contributions of the two teams of authors, especially regarding downstream operations in the production of ethanol, or the use of conventional raw materials for unconventional applications. To the opinion of the editor, this might rather be considered as an advantage according to the motto:

Duo cum faciunt idem, non est idem (Terentius).

It is anticipated that the treatise and the data presented will help readers with different scopes and professions to examine and decide whether and where the production of ethanol under a given set of conditions will be justified, and perhaps some of the facts and considerations presented might induce new ideas.

Last but not least, the editor of this volume wishes to acknowledge the excellent cooperation and patience of Karin Dembowsky from WILEY-VCH.

Vienna, October 2000 M. Roehr

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Introduction

Max Roehr
Vienna, Austria

Manufacture of alcoholic beverages is in fact as old as human civilization. The production of pure ethanol apparently begins in the 12–14th century along with improvements in the art of distilling permitting the condensation of vapors of lower boiling liquids. Wine from Italy is said to have been one of the first raw materials. During the Middle Ages, alcohol was mainly used for the production or as a constituent of medical drugs (aqua vitae, aqua ardens), but also for the manufacture of painting pigments. It was Paracelsus (1526) who coined the name alcohol or alcool. Only the knowledge of using starchy materials (cereals), first employed probably in the 12th century in typical beer making countries such as, e.g., Ireland (viske beatha) and becoming known in many countries during the next centuries, led to the flourishing trade of distillers manufacturing potable spirits. And it was only in the 19th century that this trade became an industry with enormous production figures, due to economic improvements of the distilling process, i.e. the development of the two(or more)-chambered still system, dephlegmation and rectification. In Germany, the use of potatoes as the main feedstock also contributed to this fast development. It should be mentioned that this industry was always in close connection with agricultural production. Especially in large production areas it was realized that part of the crop and crop products that could not be put on the market due to travel distances and keeping quality, was profitably converted into ethanol and marketed or used otherwise. Stillage produced as a by-product could be utilized as an excellent fodder for farm animals in the same area, and the animal excrements were equally excellent fertilizers. Thus a perfect closed-cycle economy could be established which, e.g., in former Prussia, was supported and regulated by the government. The advantages of such systems are obvious in view of the fact that in certain areas this economy was considered as fodder production with alcohol as by-product.

Although a large portion of industrial alcohol went into alcoholic beverages, further applications for ethanol were exploited, e.g., as fuel, for lighting purposes, and for various uses in the chemical industry. At the beginning of the 20th century, further raw materials were exploited, such as molasses or sulfite waste liquors, and the possibility of hydrolyzing lignocellulosic materials was investigated at several locations, for the greater part in Germany (Hägglund, Scholler; Bergius) and the USA.

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In the first half of the 20th century in particular World Wars I and II as well as the economic recession after World War I must be mentioned as incentives for further development. At the beginning of the 20th century it had become known that alcohol might be used as a fuel for various combustion engines, especially for automobiles. This, on the other hand, led to the invention of several methods for mass production of absolute ethanol. In 1906, the U.S. Congress even removed the tax on alcohol to support farmers in producing their own fuels. Ethanol was also increasingly used in the industry of lacquers and, particularly in the USA, considerable amounts of ethanol were used as anti-freeze in the automobile industry.

During the great depression of the1930s, in the USA maize was selling at less than $ 6 per ton. The USDA became active in establishing special laboratories to fund studies on the conversion of agricultural (surplus) products into useful materials. Many biotechnologists are familiar with the story of these utilities the further work and expertise of which made possible several of the most important developments in biotechnology in the 20th century, e.g., acid fermentations and penicillin production. In the ethanol field, this led to the erection of a larger plant in 1938 by Dow Chemical Company and Ford Motor Company. According to this program gasoline blends were marketed in several states of the USA. During World War II, ethanol production was part of the synthetic rubber program. After the war, caused by interventions of the U.S. petroleum industry, most of the respective plants were dismantled and sold as scrap metal.

In the early 1970s, the so-called Gasohol Program of Nebraska was founded, predecessor of the National Gasohol Program of the DOE and USDA. Professor Scheller of the University of Nebraska coined the name Gasohol. An important step in this connection was the decision of the U.S. Congress to exempt Gasohol from the motor fuel excise tax. It has been reported that the annual U.S. ethanol production can contribute US$ 1.5 billion to the trade balance. In the 1980s, Canada followed with a program similar to that of the USA.

Another milestone in large-scale alcohol technology is the Brazil ethanol program. Similar to the U.S. program, it was launched in the 1970s, but the main aim is to diminish the country's dependence on oil imports. In contrast to the U.S. program that mainly provides a 6–10% addition of ethanol, the ambition of the Brazil program was to provide a 100% ethanol fuel using sugar cane as raw material. This requires specific efforts to establish the necessary structure and size of installation as well as changes in motor design. It is reported, again, that (up to 1997) Brazil has saved more than US$ 35 billion in foreign exchange through reduced oil imports.

Beginning in the early 1980s and continuing since then, several countries, especially in Europe, have decided to initiate programs for larger-scale production of ethanol from indigenous materials. The motivations are rather divergent, but the main aims are to subsidize agricultural production, to stabilize the trade balance and, increasingly, to consider the necessity of environmental pro-

tection. It becomes apparent that development in biotechnology is not only determined by scientific and technological innovation, but more and more by a number of external forces: Prices of raw materials have to be compared with that of petroleum or ethylene considering at least actual costs and availability of foreign exchange. Political (ideological) conditions may influence decisions on various levels difficult to anticipate. Increasingly, requirements of environmental protection have to be considered and may demand changes in process technology as well as the employment of either renewable feedstocks or waste materials.

In summarizing, it becomes more and more apparent that there are rather complex sets of conditions determining whether alcohol can be produced and marketed economically (and ecologically). It is the objective of the present book to provide data and considerations towards an objective judgement of a complex area of classical and modern biotechnology.

Part I

The Biotechnology of Ethanol: Classical and Future Applications. Edited by M. Roehr

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Classical Methods

Thomas Senn and Hans Joachim Pieper
Stuttgart, Germany

1 Starch Containing Raw Materials

1.1 Potatoes

Potatoes represent the most widely used starchy raw material in ethanol production in Germany and Eastern Europe. On average potatoes consist of ca. 75% water and 25% dry substance. An average analysis is given in Tab. 1. Regarding the suitability for ethanol production, the starch content of potatoes is the most important criterion. In addition to starch, potatoes contain low amounts of sugars, mainly sucrose, glucose, and fructose. Sugar and starch contents depend on the variety and level of ripeness of the potatoes. The starch content also depends on climatic, growth, as well as on storage conditions. The loss of starch during storage, e.g., amounts to about 8% after 6 months and 16.5% after 8 months of storage in a regular operating storage cellar.

As shown in Tab. 1, potatoes contain pectin. This pectin content is responsible for the methanol content of spirits produced from potatoes. Milling of potatoes leads to the release of pectin esterases which immediately start cleavage of the methyl ester bonds of pectin. Using a pressure cooking process in mashing potatoes, pectin esterases are inactivated by heat, but a virtually complete thermal de-esterification takes place. This is why the methanol content in raw spirits obtained from pressure cooking processes is higher compared to raw spirits produced by pressureless processes (Boettger et al., 1995).

Tab. 1. Average Analysis of Potatoes

Component	Percentage [%]
Water	72.0 –80.0
Starch	12.0 –21.0
Sugar (reducing)	0.07– 1.5
Dextrin and pectin	0.2 – 1.6
Pentosans	0.75– 1.0
Nitrogenous components	1.2 – 3.2
Fat	0.1 – 0.3
Crude fiber	0.5 – 1.5
Ash	0.5 – 1.5

1.2 Wheat

Wheat is often used in German grain distilleries, because it yields an especially mild and smooth distillate. The starch content of wheat is usually about 60%, leading to ethanol yields of about 38 lA per 100 kg wheat (Tabs. 2 and 3). If wheat containing more than 13% raw protein is used for ethanol production, fermentation problems may occur. If wheat with a high protein content is processed without pressure, the mashes tend to foam during fermentation. Often these mashes can only be fermented if an antifoam agent (e.g., silicone antifoam) is used in fermentation. Tabs. 2 and 4 show the composition of wheat grains.

The possibility of using a certain pressureless processing of wheat depends on the activity of the autoamylolytic enzyme system, which can be measured by determination of the autoamylolytical quotient (AAQ) (see Sect. 13.2.3). Fig. 1 shows the AAQ of several important varieties of wheat. The pressureless processing of wheat is possible without any problems if the lot of wheat used has an AAQ of 95% or higher. In general, the processing of waxy wheat is problematic and requires a very effective decomposition of the raw material (Sect. 8.1).

Tab. 2. Components of Wheat Grains

Component	Percentage [%]
Seed coat	15.0
Endosperm	83.0
Germ	2.0

Tab. 3. Average Analysis of Wheat

Component	Percentage [%]
Water	13.2
Crude protein	11.7
Crude fat	2.0
NNE	69.3
Crude fiber	2.0
Ash	1.8

Tab. 4. Composition of Wheat Components [% of DS]

Component	Protein	Ash	Fat	Carbohydrates
Seed coat	7–12	5 – 8	1	80–88
Aleuron layer	24–26	10 –12	18 –10	52–58
Endosperm	10–14	0.4– 0.6	1.8– 1.2	83–87
Germ	24–28	4 – 5	8 –12	55–64

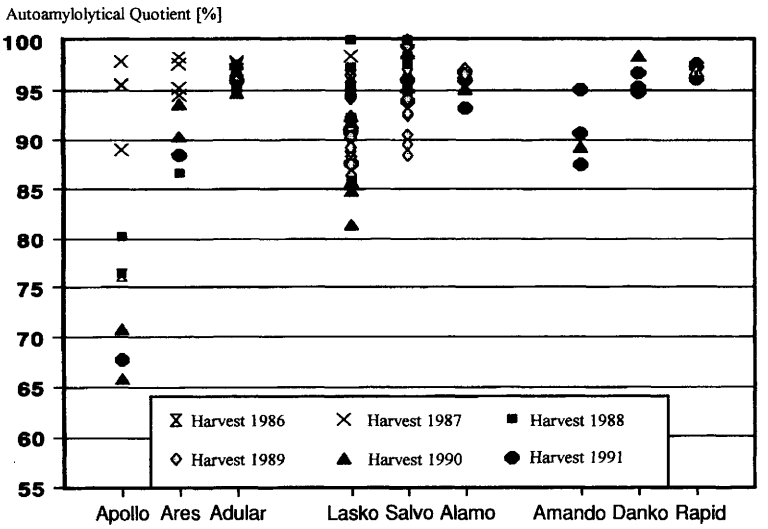

Fig. 1. Autoamylolytical quotient of wheat, triticale and rye varieties, depending on the year of harvest.

1.3 Rye

The starch content of rye is about 2–4% lower than that of wheat. A batch of rye with a high starch content yields about 37 lA per 100 kg of rye. Rye is also an important raw material in German grain distilleries. An average analysis is given in Tab. 5.

Virtually all varieties of rye contain a highly active autoamylolytical enzyme system (Fig. 1) and they are, therefore, suitable for pressureless processes. However, as shown in Tab. 5, rye may contain pentosans, depending on climatic and growth conditions as well as on variety. These pentosans often lead to high viscosities in rye mashes, resulting in problems during the mashing and the fermentation (Sect. 8.3).

Tab. 5. Average Analysis of Rye

Component	Percentage [%]
Water	13.7
Crude protein	11.6
Crude fat	1.7
NNE	69.0
Crude fiber	2.1
Ash	1.9

1.4 Triticale

Triticale, a hybrid of wheat and rye, which has been used in ethanol production since just a few years, is a very important raw material. The starch content is about 60% of original substance leading to ethanol yields of 38 lA per 100 kg of triticale. Triticale does not contain considerable amounts of pentosans, and so there are no problems regarding mash viscosity.

Some varieties of triticale exhibit high autoamylolytical enzyme activity and, therefore, it is possible to process triticale without using any additional saccharifying enzyme. To examine single lots of triticale, it is necessary to determine the AAQ as described in Sect. 13.2.3. Not only is it possible to saccharify the starch in triticale, but the same amount of starch from other grains or from potatoes can additionally be saccharified. Triticale is a potentially rich source of the saccharifying enzymes needed in distillery. The composition of triticale has rarely been examined; the data shown in Tab. 6 are approximations.

1.5 Corn (Maize)

1.5.1 Dried Storable Corn Grain

Corn is a very important raw material for ethanol production in the USA and South America. In Europe great amounts of corn are also used for ethanol production. An averarge analysis is shown in Tab. 7. The suitability of corn for ethanol production depends on the contents of starch and horny endosperm. A high content of horny endosperm leads to problems in ethanol production using milling processes (Sect. 5.4.1). European corn, with a starch content of about 62–65%, yields at least 40 lA per 100 kg of corn. Tab. 8 shows a typical analysis of corn grain from a Southern German distillery. When purchasing corn, its water content and cleanness must be considered. The fat contained in corn prevents foam formation during the fermentation.

Tab. 6. Composition of Triticale (Kling and Wöhlbier 1983)

Component of DS [%]	
OS	97.2
Crude protein	17.3
Crude fat	1.8
Crude fiber	3.1
NNE	73.8
Ash	2.3

Tab. 7. Average Analysis of Corn Grain [% of DS] (Kling and Wöhlbier, 1983)

Component	Naturally Dried Mean Value (n = 496)	Artificially Dried Mean Value (n = 12)
	Mean s	Mean s
OS	98.3 ± 0.6	98.2 ± 0.6
Crude protein	10.8 ± 1.1	10.7 ± 1.0
Crude fat	4.7 ± 0.8	4.7 ± 0.6
Crude fiber	2.6 ± 0.8	2.6 ± 0.3
NNE	80.2 ± 2.2	80.2 ± 1.2
Ash	1.7 ± 0.6	1.8 ± 0.6
DS	ca. 88	88.9 ± 2.9

Tab. 8. Average Analysis of Dried Storable Corn Grain from a Distillery in Southern Germany (Pieper and Pönitz, 1973)

Component	Minimum [%] of OS	Maximum [%] of OS	Mean Value [%] of OS n = 3
Water	14.8	15.2	15.1
Ash	1.4	1.5	1.5
Crude protein	8.3	8.5	8.4
Crude fiber	1.9	2.1	2.0
Fat	3.6	3.9	3.7
Starch	62.2	63.1	62.6
Other NNE	6.3	7.4	6.7

1.5.2 Corn Grain Silage

In many parts of Europe, due to climatic conditions, corn does not ripen sufficiently to be harvested as natural dried corn grain. Very often corn grain has to be dried artificially which leads to high costs for drying. These costs can be re-

Tab. 9. Average Analysis of Corn Grain Silage form a Distillery in Southern Germany (Pieper and Pönitz, 1973)

Component	Minimum [%] of OS	Maximum [%] of OS	Mean Value [%] of OS n = 6
Water	41.6	41.6	41.6
Ash	1.0	1.1	1.1
Crude protein	5.7	5.8	5.8
Crude fiber	1.4	1.7	1.6
Fat	2.4	2.6	2.5
Starch	42.3	42.5	42.4
Other NNE	5.0	5.5	5.0

duced if corn is used in distilleries as corn grain silage (Pieper and Pönitz, 1973). Corn grain silage can effectively be processed using HPCP or better using DMP with stillage recycling (Sect. 5.4.2). A typical analysis of corn grain silage from a distillery in Southern Germany is shown in Tab. 9.

If corn grain silage is used in ethanol production, one has to take care that a pure lactic acid fermentation takes place. Minimal concentrations of butyric acid in the stillage, due to a contamination of silage with butyric acid bacteria, lead to a total breakdown of the fermentation since butyric acid is strongly toxic for yeasts.

1.6 Barley

In ethanol production, barley is mostly used as malting grain. Since it grows very well in Eastern Europe, it is also an interesting raw material in ethanol production. There are two notable disadvantages of barley as a raw material in distilleries: the husks surrounding the kernels and the content of glucans which leads to high viscosities in mashes. Therefore, special processing is necessary in preparing mashes from barley (Sect. 5.5). Tab. 10 shows an average analysis of barley. Consisting of about 55% starch, barley yields about 35 lA per 100 kg FS. Compared to distillates from wheat, potable distillates produced from barley are smooth, but have a more powerful grain taste.

1.7 Sweet Sorghum

Sweet sorghum is rarely used in Europe for ethanol production. About its composition there are no reliable data available in the literature regarding ethanol production. But within the last 20 years the growth of sweet sorghum for

Tab. 10. Average Analysis of Barley [% of DS] (Kling and Wöhlbier, 1983, modified)

Component	Mean Value (n = 1249) Mean s
OS	97.2 ± 0.7
Crude protein	11.8 ± 1.7
Crude fat	2.2 ± 0.5
Crude fiber	5.3 ± 1.5
NNE	77.9 ± 2.6
Starch as a part of it	63.2
Ash	2.8 ± 0.7
DS	ca. 87%

ethanol production in Austria and Germany has been investigated (Salzbrunn, 1982; Diedrich et al., 1993). Sweet sorghum is a native plant of subtropical and tropical regions, but also grows in certain parts of Austria and Germany reaching a height of 3–3.5 m. Trial plots yielded 5–8.8 t of fermentable sugars per ha, depending on the cultivation site. To obtain the sugar-containing juice from the sweet sorghum plant it can either be extracted with water or it can be pressed out using roller mills. For conservation it can be concentrated up to 80v Bx using a downflow evaporator (Salzbrunn, 1982).

1.8 Sorghum Grain

The worldwide production of sorghum grain takes the fourth place of all varieties of grains. Sorghum grain exists in yellow and brown colored types that show no significant differences in composition. An average analysis of sorghum is shown in Tab. 11. Sorghum kernels are round with a diameter of 5–7 mm. Sorghum contains about 62–65% starch and yields about 40 l A per 100 kg of sorghum. These data are comparable to corn.

When purchasing sorghum grain, one should check its cleanness, especially it should be free from sand and corn weevils. Due to the fact that sorghum starch is waxy, it is not easily decomposed. Therefore, sorghum should either be processed with HPCP, or preferably by DMP and stillage recycling.

1.9 Manioc

Manioc is a tropical plant, forming starch-containing roots. Since manioc roots do not keep well, they should be processed immediately or, alternatively manioc starch can be produced from dried roots. It is also possible to mill the roots and to dry the obtained manioc flour. Average analyses of different manioc products are given in Tab. 12.

Tab. 11. Average Analysis of Sorghum Grain

Component	Percentage [%]
Water	11–12
Crude protein	9–12
Fat	3–4.5
NNE	up to 71
Crude fiber	3
Ash	1.5–3
Starch and sugar	58 –63

Tab. 12. Average Analysis of Manioc Products

Component	Manioc Root	Manioc Starch	Manioc Flour
Water	70.3	12.6	14.0
Protein	1.1	0.6	1.2
Fat	0.4	0.2	0.4
Fiber	1.1	0.2	2.0
Starch	21.5	–	74.3
Ash	0.5	0.3	1.4
Non-nitrogenous components (including starch)		86.1	81.0

Manioc contains toxic levels of a cyanogenic glucoside (up to 2.8 g per kg DS), and, therefore, it is recommended in ethanol production to process manioc using HPCP. In this way manioc products are detoxified by deaeration. It is also possible to detoxify manioc products by adding sodium thiosulphate, and hence it should be possible to process manioc using other mashing processes. There are increasingly more varieties of manioc being cultivated which are free from cyanogenic glucosides.

Sometimes manioc flour or manioc starch contain up to 20% sand. If this is not detected before the material is processed, the sand will settle down in the fermentation tank and take the yeast with it, leading to drastical disruptions during fermentation (Kreipe, 1982).

2 Technical Amylolysis

To reach an almost total degradation of starch to fermentable sugars in technical processes, two main groups of amylolytical enzymes are required: one group comprisesliquefying α-amylases, the other group saccharifying glucoamylases, β-amylases, and α-amylases.

2.1 Enzymatic Starch Liquefaction

Technical liquefying enzymes are virtuallyall α-amylases (α-1,4-glucane4-glucanohydrol-ase, E.C. 3.2.1.1) that split α-1,4 bonds in amylose and amylopectin. α-Amylase is an endo-acting enzyme and its action is often considered to be random, i.e., the enzyme has equal preference for all α-1,4 linkages except those adjacent to the ends of the substrate chain and those in the vicinity of branch points. The α-1,6 glycosidic bonds are not hydrolyzed. The properties as well as the action of α-amylase depend on the microorganisms or plants from which it is derived. However, all α-amylases rapidly decrease the viscosity of starch solutions.

2.1.1 Thermostable Bacterial α-Amylase of *Bacillus licheniformis* (TBA)

TBA was isolated, purified, and characterized by Chiang et al. (1979). The characteristics of TBA in this work were determined with soluble lintner starch as substrate. The optimum pH for the purified enzyme is between 6 and 7 as shown by Chiang et al. (1979), the optimum temperature is 85 °C. It was further shown, that upon hydrolyzing corn starch with TBA, mainly maltotriose, maltopentaose, and maltohexaose were formed. Without substrate and without added calcium ions stability of TBA decreases rapidly at temperatures above 65 °C. Consequently TBA may be added during mashing processes only when the substrate is present. Using a technical enzyme preparation of α-amylase from *B. licheniformis*, Rosendal et al. (1979) also showed that the optimum pH for the hydrolysis of soluble starch by TBA lies between 6 and 7. The enzyme used in this work was absolutely stable at 90 °C. An investigation of the action of technical amylolytic enzymes using corn mash as substrate was described by Senn (1988). An optimum pH range from 6.2 to 7.5 was found, with pH values below 5.6 leading to a rapid decrease in enzyme activity. The optimum temperature for TBA in this work was 80–85 °C. Furthermore, it could be shown that enzyme activity also depends on the proportion of horny to floury endosperm of the processed corn. The higher the proportion of horny endosperm, the lower the enzyme activity determined in such mashes. This shows that it is more difficult to digest starch from horny than from floury endosperm.

Liquefaction of corn mashes using TBA yields mainly starch fragments with a degree of polymerization of more than 10 glucose units as well as maltose and glucose. But the content of glucose and maltose does not rise to more than 5 g L^{-1} mash for each component after 30 min of liquefaction. During 4 h of liquefaction there is no further progress in degradation. If fermentable sugars are metabolized by fermentation, the starch fractions DP 4 to DP 7 rise, but fermentable sugars can not be determined (Senn, 1992).

2.1.2 Bacterial α-Amylase of Bacillus subtilis (BAA)

Determined in soluble starch as substrate BAA shows an optimum pH value between 5.3 and 6.4, and an optimum temperature of 50 °C (Robyt, 1984).

Fogarty and Kelly (1979) reported that with starch as substrate BAA produces doubly branched limit dextrins. Furthermore, two highly branched dextrins containing 9 and 10 glucose units were isolated, and both were shown to be mixtures of 4 triply branched dextrins. These low molecular branched limit dextrins are very difficult to hydrolyze with glucoamylase from *Aspergillus niger*. That is why starch degradation often remains incomplete if BAA is used for liquefaction.

Using corn mash as substrate the optimum conditions for BAA are a pH between 5.8 and 6.8 and a temperature of 55–60 °C (Senn, 1988; Senn and Pieper, 1991). Under these conditions BAA is stable up to 65 °C if the pH is adjusted to between 6.2 and 6.4.

2.1.3 Bacterial α-Amylase Expressed by *Bacillus licheniformis* (BAB)

BAB, a new technical enzyme produced with a genetically engineered strain of *B. licheniformis* (Liquozym, NOVO Nordisk, Denmark) has been available for the past years (Klisch, 1991). BAB is characterized by its tolerance to low pH values down to 4.8–4.5. But it is only possible to liquefy cereal mashes using BAB. Liquefaction of cereal mashes is very effective; in mashes from potatoes it works insufficiently. This enzyme is thermostable up to 90 °C. Due to its pH tolerance, BAB is the optimum liquefaction enzyme in processes with included recycling of stillage.

2.1.4 Fungal α-Amylase of *Aspergillus oryzae* (FAA)

As reported by Fogarty and Kelly (1979), FAA contains only a few amino acid residues. Therefore, FAA is relatively stable in the acid pH range. The optimum conditions for this enzyme have been reported to be a pH value between 5.5 and 5.9 and a temperature of 40 °C. Depending on the stability of FAA the pH value can range from 5.5 to 8.5 (Fogarty and Kelly, 1979). Furthermore, as reported by Takaya et al. (1978), FAA is able to attack native starch granules. At a pH of 7.2 and a temperature of 37 °C after 60 h more than 40% of starch weighed in was dextrinized.

Using corn mash for determination of enzyme properties and under technical conditions, the optimum pH ranges from 5.0 to 6.0. At a pH of 4.5, FAA displays 50% of its activity measured under optimum conditions (Senn, 1988). The optimum temperature is reported to lie within the range of 50–57 °C.

The use of FAA promotes a quite effective further decrease in viscosity at the saccharification temperature combined with a more effective dextrinization of starch. This supports a total degradation of starch. The pH tolerance of FAA guarantees that the enzyme, for a certain time, is active during the fermentation until the pH falls below 4.5.

2.2 Enzymatic Starch Liquefaction and Saccharification

Malt is the classical source of amylolytical enzymes used in alcohol production technology. It contains both liquefying and saccharifying enzymes. The amylolytical components of malt are

- α-amylase (Sect. 2.1),
- β-amylase (α-1,4-glucan maltohydrolase, EC 3.2.1.2),
- limit dextrinase,
- *R*-enzyme.

As reported by Sargeant and Walker (1977), α-amylase from malt can hydrolyze native starch granules.

β-Amylase is an exo-acting enzyme and hydrolyzes starch yielding maltose. Starch molecules are attacked from the non-reducing end of the glucose chains. β-Amylase hydrolyzes only α-1,4 linkages and is unable to bypass α-1,6 glycosidic linkages in amylopectin. Degradation of branched amylopectin, therefore, is incomplete. Action of β-amylase on amylopectin results in a 50–60% conversion to maltose and the formation of β-limit dextrin containing all α-1,6 linkages. The optimum conditions for β-amylase are a temperature of 50 °C and a pH of 5.0. β-Amylase is stable within a pH range of 4.0–6.0.

Limit dextrinase from malt has an optimum pH of 5.1 and an optimum temperature of 40 °C. This enzyme is unable to cleave α-1,6 linkages in substrates that do not contain a sufficient number of α-1,4 linkages. Limit dextrinase is also unable to dextrinize amylopectin or β-limit dextrin; it mainly debranches and dextrinizes α-limit dextrins. It was shown by Harris (1962) that there is a higly significant correlation between ethanol yield using pressureless processes and the limit dextrinase content of the malt used.

Another debranching enzyme from malt is the *R*-enzyme. This enzyme cleaves α-1,6 linkages in amylopectin and β-limit dextrin; α-limit dextrins are not attacked by *R*-enzyme. Debranching of amylopectin and β-limit dextrin is, however, incomplete, since the *R*-enzyme needs 5 or more glucose units between two α-1,6 linkages in order to cleave them. The optimum conditions for *R*-enzyme are 40 °C and a pH of 5.3 (Harris, 1962).

All of these amylolytical enzymes from malt work together and act very fast. After only 15 min of action on the substrate a maltose–dextrin equilibrium is reached with about 66% maltose, 4% glucose, 10% maltotriose, and 20% limit dextrins. Saccharification is not completed during the mashing process using exclusively malt. Saccharification is only completed if maltose is metabolized by yeast fermentation. Hence, there are two steps in saccharification: a first step of the main saccharification reaching an equilibrium and a second step of residual saccharification during the fermentation ("secondary fermentation").

2.2.1 Green Malt

The use of green malt, manufactured in distilleries, for liquefaction and saccharification in classical distillation technology has a long tradition. It was often used in potato distilleries up to 1970. Nowadays, since technical enzyme

preparations are available commercially, the use of green malt is too expensive. However, it is of great interest for Eastern European countries with limited foreign currency reserves where technical enzymes are not available, and barley is grown without any problems.

The malting process in distilleries comprises two main stages: the steeping of barley and the germination process. Both of these production steps can be carried out in one apparatus by using a Galland malting drum (Narziss, 1976; Schuster, 1962; Lewis and Young, 1995). This pneumatic malting system, which can take a batch size of up to 15 t of barley, is the optimum related to the needs of distilleries.

In order to use green malt in distilleries, it is necessary to grind it thoroughly. A special apparatus (Kreipe, 1981) is in use to produce the malt slurry with a green malt to water ratio of 1:3. This apparatus consists of a vessel which is filled up with the required amount of water. A centrifugal pump, fitted as a centrifugal mill, recirculates the water and the malt slurry. Green malt is then added to the water, avoiding clots. Methanal (formaldehyde) may also be added for disinfection. The malt slurry is thoroughly ground after 30–40 min.

2.2.2 Kiln-Dried Malt

To produce a distillers' kilned malt, it is important to use low temperatures in kilning to save enzyme activity. This reduces the moisture content of green malt initially to 10–12% by passing a large excess of air for 12 h at a temperature of 40–50 °C through the grain. Subsequently the moisture content is further reduced to 4–5% by raising the air temperature to 55–60 °C.

Kilned malt must be thoroughly ground before use in a mashing process. The milled malt is then mixed with water in a ratio of 1:3 and at 50 °C to bring enzymes into solution. Methanal (formaldehyde) may be added for disinfection.

The α-amylase activity of malt is determined by the SKB method, according to Sandsted, Kneen, and Blish (Pieper, 1970). Drews and Pieper (1965) recommend the evaluation presented in Tab. 13.

2.2.2.1 Barley as a Malting Grain

Barley is the most widely used grain in malting. Barley used for making distillers' malt is of smaller size and higher nitrogen content than barley used for brewer's malt. Before malting, barley must be cleaned and free from weed seeds and broken grains. For steeping, barley usually is treated in water twice for 2–4 h, and exposed to air for 20–24 h after each steeping. The temperature is adjusted to 10–12 °C, and must not exceed 15 °C. The germination period lasts for 6–8 d and the grain is turned twice daily (Schuster, 1962). By adding

Tab. 13. Evaluation of Dried Distillers' Malt (Drews and Pieper, 1965)

Activity of α-Amylase SKB-Units per g Malt DS	Evaluation
>64	excellent
53–64	good
41–52	sufficient
<41	insufficient

gibberellic acid to the barley when the germination period is started, the germination time can be reduced to 4–6 d while reaching the same or higher enzyme activities (Pieper, 1968).

2.2.2.2 Other Grains in Malting

Wheat can also be very effectively used as a malting grain (Pieper, 1984). The batches of kiln dried wheat malt examined in this work exhibited α-amylase activities of between 117 and 165 SKB-units per g of malt DS, which is three times the activity of a good kilned barley malt. The ethanol yield of wheat malt, which is more than 38 lA per 100 kg malt, is impossible to reach with barley malt. Pieper (1984) further found that the use of wheat dried malt for saccharification of wheat mashes yields 67 lA per 100 kg of starch. The wheat-to-malt ratio was 10:1. The excellent ethanol yield was reached by keeping a saccharification rest of 30 min at 55 °C and a pH of 5.5. There are, however, some difficulties in malting wheat, especially during the germination stage. Germination is manifested by the growth of roots and the shoot. The growing roots have a tendency to break easily, leading to losses in enzyme activity and increasing the risk of infections during germination. This is probably the reason why wheat is generally not used in manufacturing distillers' malt.

Triticale is another grain which can very effectively be used in malting for distillers use (Thomas, 1991). To reach optimum enzyme activities, triticale was steeped at 15 °C for 48 h. Therefore, a pneumatic malting system was used, and triticale was steeped in water twice for 4 h. For the rest of the time triticale was aerated and sprinkled with water at 15 °C. At the end of this steeping procedure, the tip of the root was just breaking through the husk, and the water content was 38–42%. Triticale was then transferred to the germination chamber and allowed to germinate at 15 °C. During germination triticale was aerated with humidified air. Kilning was carried out at temperatures between 40 °C and 50 °C.

After malting under these conditions, α-amylase activities in triticale malts reached 170 SKB-units per g of malt DS after only 4 d of germination. The optimum conditions for the use of triticale malt in saccharification are 55 °C and

a pH of 5.2–5.5. By saccharifying corn mashes using kilned triticale malt (with a corn-malt ratio of 10:1), ethanol yields from about 67 lA per 100 kg starch could be reached. These yields of ethanol from corn cannot be obtained with using barley malt. Hence, triticale is a very important grain in malting for distillers' use.

2.3 Enzymatic Starch Saccharification

Glucoamylase (EC 3.2.1.3) is an exo-acting enzyme, hydrolyzing α-1,4 α-1,6, and α-1,3 glycosidic linkages in amylose and amylopectin. The rates of hydrolysis depend on the molecular size and structure of the substrates (Fogarty and Kelly, 1979). Thus glucoamylase from *Aspergillus niger*, e.g., hydrolyzes isomaltose at a lesser rate than maltose: These authors show, that branched substrates are hardly degraded by glucoamylases derived from several fungi. This may be a problem in alcohol production technology, if an α-amylase is used for liquefaction that yieldsdouble or triple branched α-limit dextrins.

2.3.1 Glucoamylase of *Aspergillus niger* (GAA)

Two structurally different glucoamylases from *Aspergillus niger*, glucoamylase 1 and glucoamylase 11, have been characterized (Fogarty and Kelly, 1979). The enzymes differ mainly in amino acid composition. Both enzymes, examined with soluble starch as substrate, were found to have a pH optimum of 4.5–5.0 and an optimum temperature of 60 °C; the isoelectric point is given for GAA1 as 3.4, and for GAA 11 as 4.0.

Using corn mash as substrate, the optimum range of pH value reaches from 5.0 down to 3.4 (Senn and Pieper, 1991; Labeille et al., 1997). Thus, GAA is stable during fermentation. With respect to temperature, GAA in this work was found to be stable up to 70 °C with an optimum at 65 °C.

2.3.2 Glucoamylase of *Rhizopus* sp. (GAR)

The optimum conditions for GAR are a temperature of 40 °C and a pH value ranging from 4.5 to 6.3 (Fogarty and Kelly, 1979). The manufacturers of technical GAR products report the optimum conditions as 40–60 °C and a pH range from 4.0 to 5.5.

Two kinds of glucoamylases were isolated from a *Rhizopus* sp. Glucoamylase 1 shows strong debranching activity and is able to degrade raw starch, while glucoamylase 11 generally shows low activities in both cases. This special debranching activity of glucoamylase 1 from a *Rhizopus* sp. is very useful in

achieving an almost total conversion of starch to fermentable sugars in pressureless processes.

Using corn mash as substrate, the optimum conditions for GAR were found to be 55–60 °C and a pH of 4.4–5.4 (Senn and Pieper, 1991); the enzyme was stable at a pH as low as 3.8. To save supplementary hemicellulolytic and proteolytic activities in technical GAR preparations, the temperature of mashes should not be higher than 52 °C when the enzyme is added.

2.3.3 Enzyme Combinations

In practice, single enzymes are rarely used for saccharification of mashes. Due to the different characteristics of the various enzymes, it is important to know which enzymes may be combined successfully in mashing processes and fermentation. As reported by Senn (1992), different combinations of technical enzymes may exhibit either complementary or inhibitory effects. To examine these effects, starch degradation in corn mashes was followed using several technical enzyme combinations. During mashing and fermentation processes the content of saccharides and oligosaccharides up to DP10 (degree of polymerization) in mashes was measured by HPLC. The mashing processes in these examinations where carried out with saccharification rests of about 30 min, 360 min, and without any saccharification rest.

With a saccharification rest of 6 h, the combination of GAA and FAA, often used in practice, leads to a rapid degradation of the fraction with high molecular weight, with a rapid increase in glucose and maltose concentrations. However, further degradation of the fraction DP > 10 is quite slow and remains incomplete. After 24 h of fermentation, the amounts of fermentable sugars are very low, resulting in a slow and incomplete saccharification and fermentation (Fig. 2).

If the saccharification rest lasts for only 30 min, the maltose and DP > 10 fractions increase again during the second day of fermentation, leading to a sluggish fermentation, too.

The combined saccharification with GAR and FAA gives a significantly better degradation of the DP > 10 fraction than GAR alone (Fig. 3). The additional use of GAR together with GAA and FAA shows an inhibition, because degradation of starch is significantly slower than with the supply of GAA and FAA (Fig. 4.5).

"OPTIMALT®" (Solvay Enzymes, Nienburg) is an enzyme combination containing GAR, kiln dried distillers barley malt (DBM), GAA, and FAA, developed at the Versuchs- und Lehrbrennerei, Hohenheim University. When it is used in saccharification, the concentration of fermentable sugars in mashes rises rapidly; this is never achieved with other enzyme combinations (Fig. 6).

Even without a saccharification rest, the mashes contain sufficient amounts of fermentable sugars during the whole fermentation. Although the amount of

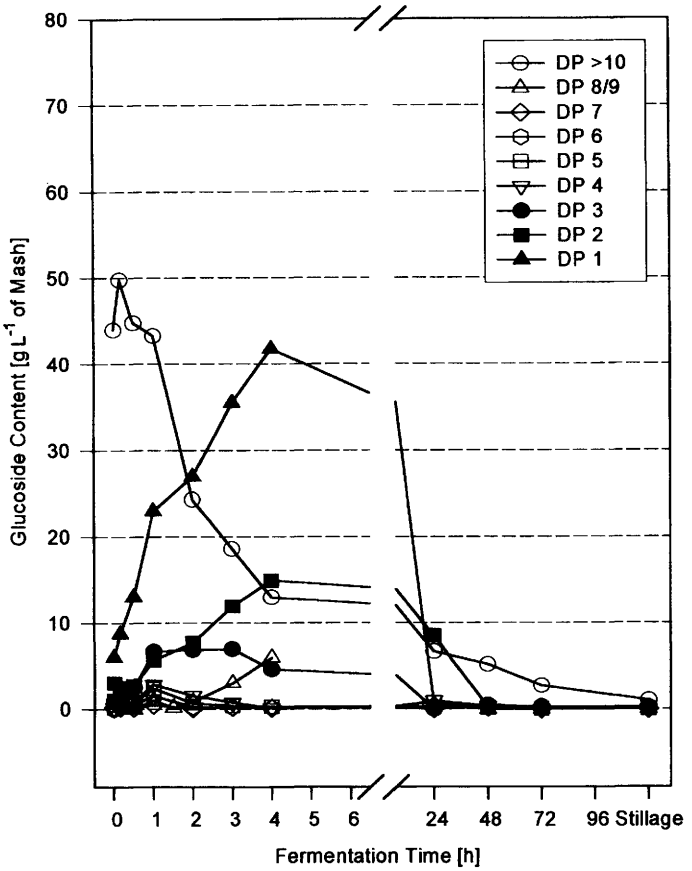

Fig. 2. Saccharification of corn mash using a combination of GAA and FAA. Yeast added after 6 h of saccharification; distillation after 72 h of fermentation.

DBM in this combination is only 3 kg t^{-1} starch, it has the same effect as when DBM is used alone; after a saccharification period of only 4 h the DP 1–DP 3 fractions are present in significant amounts. Hence, OPTIMALT® ensures a fast and almost complete degradation of starch during saccharification and fermentation, even without any saccharification rest (Fig. 7).

3 Starch Degradation by Autoamylolysis

It has long been shown that some native cereal grains (wheat, rye) contain autoamylolytic activities. These enzyme activities were often used in the traditional pressureless process called "cold mash process (Kaltmaischverfahren)" prior to 1940 (Sect. 4.3.1.1). Nevertheless, it was impossible to develop a

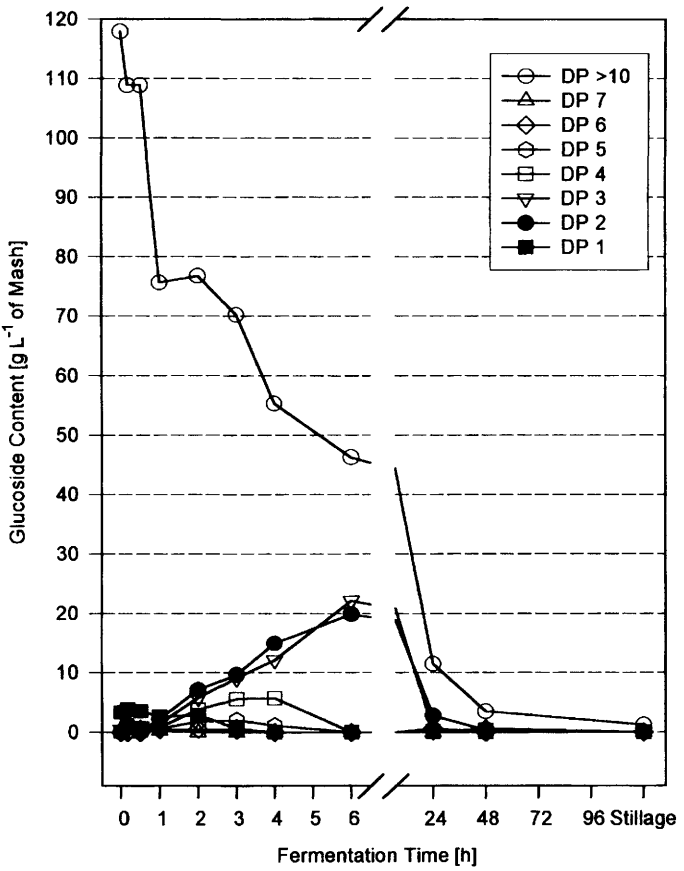

Fig. 3. Saccharification of corn mash using a combination of GAR and FAA. Yeast added when saccharification is started; distillation after 96 h of fermentation.

reliable technical process using these autoamyloytical activities due to the lack of reliable quantitative methods for the determination and examination of autoamylolytic acitivities in different charges of raw materials. Such methods (Sect. 13.2.3) were developed in 1989 at Hohenheim University, reliable technical processes using autoamylolytical activities have also been developed (Sect. 8).

To examine the autoamylolytical activity of raw materials, Rau et al. (1993) defined theso-called Autoamylolytical Quotient (AAQ). This AAQ is determined by carrying out two separate fermentation tests with the same raw material. The first fermentation test runs using technical enzymes to determine the maximum ethanol yields obtainable with the raw material used. The second fermentation test is carried out without the addition of technical enzymes or malt to determine the ethanol yield obtained under autoamylolytic

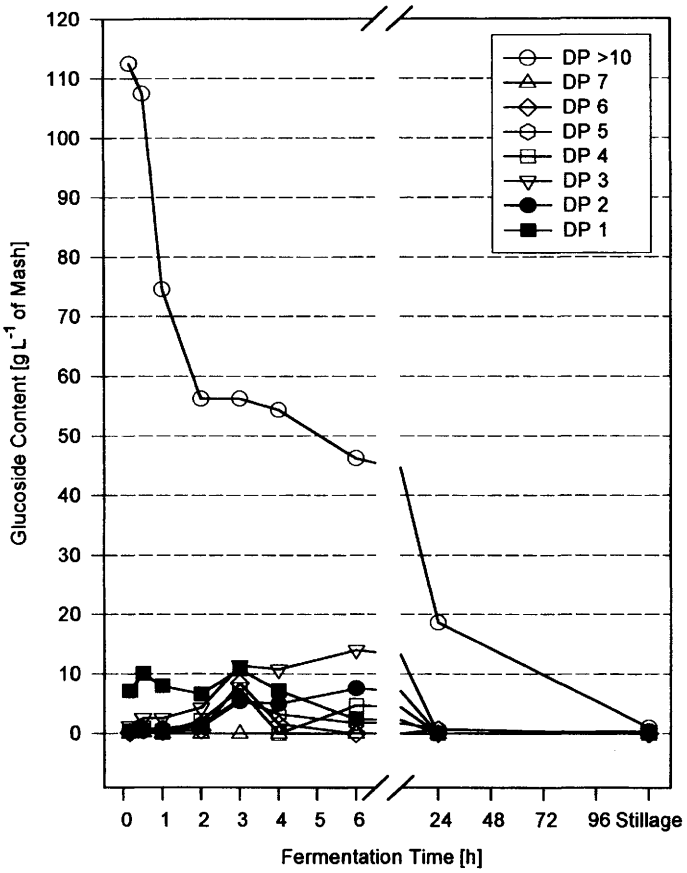

Fig. 4. Saccharification of corn mash using a combination of GAR, GAA and FAA. Yeast added after 30 min of saccharification; distillation after 72 h of fermentation.

conditions. AAQ then is, related to the raw material used, defined following Eq. (1):

$$\text{AAQ} = \frac{\text{Ethanol yield [lA/100 kg FS] without technical enzymes} \cdot 100}{\text{Ethanol yield [lA/100 kg FS] using technical enzymes}} \qquad (1)$$

The mashes used in the fermentation tests with additional technical amylases were liquefied at 65 °C using thermostable α-amylase from *Bacillus licheniformis*. This is not feasible when mashing under autoamylolytic conditions, since the autoamylolytical enzyme system does not persist at 65 °C (Rau, 1989). Total gelatinization of starch in these mashes is required to reach a complete degradation of starch to fermentable sugars, and gelatinization requires a temperature of about 65 °C, e.g., in wheat mashes. This problem can be solved by us-

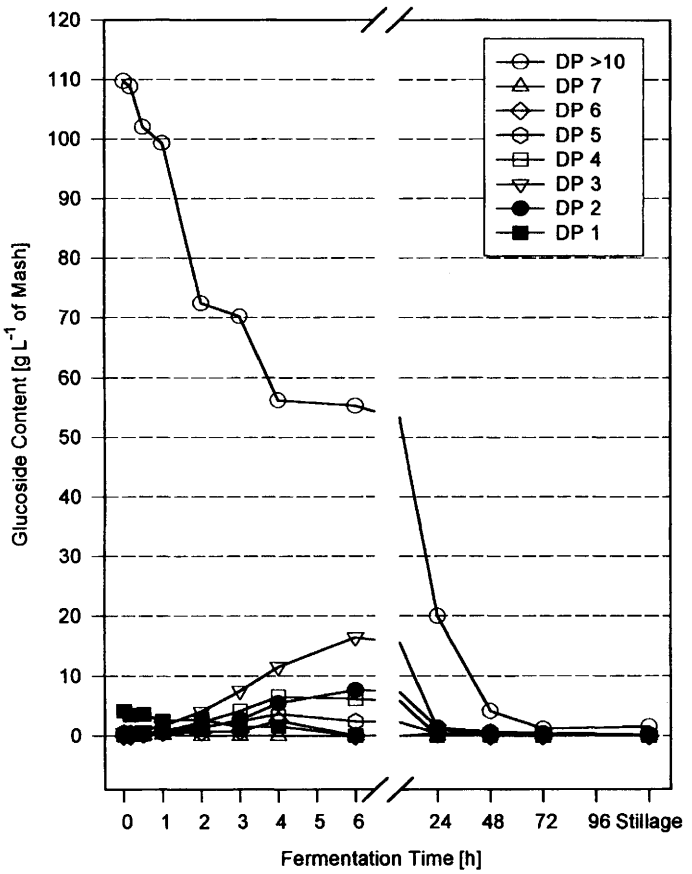

Fig. 5. Saccharification of corn mash using a combination of GAR, GAA and FAA. Yeast added when saccharification is started; distillation after 72 h of fermentation.

ing a certain time and temperature program in mashing processes, which depends on the raw material. The enzymes in native wheat kernels which are dried for storage are α-amylases, β-amylase, and limit dextrinase (Marchylo et al., 1984; Laberge and Marchylo, 1983; Manners and Sperra, 1966).

3.1 Wheat

The optimum conditions for the wheat autoamylolytic enzyme system are 55 °C and apH of 5.3–5.5 (Rau, 1989). Due to the gelatinization temperature of wheat starch, wheat mashes must be heated to 64 °C. To protect the enzyme system during the mashing process this temperature may be kept for only 10 min. Then the mash must be cooled down to 55 °C for a saccharification rest

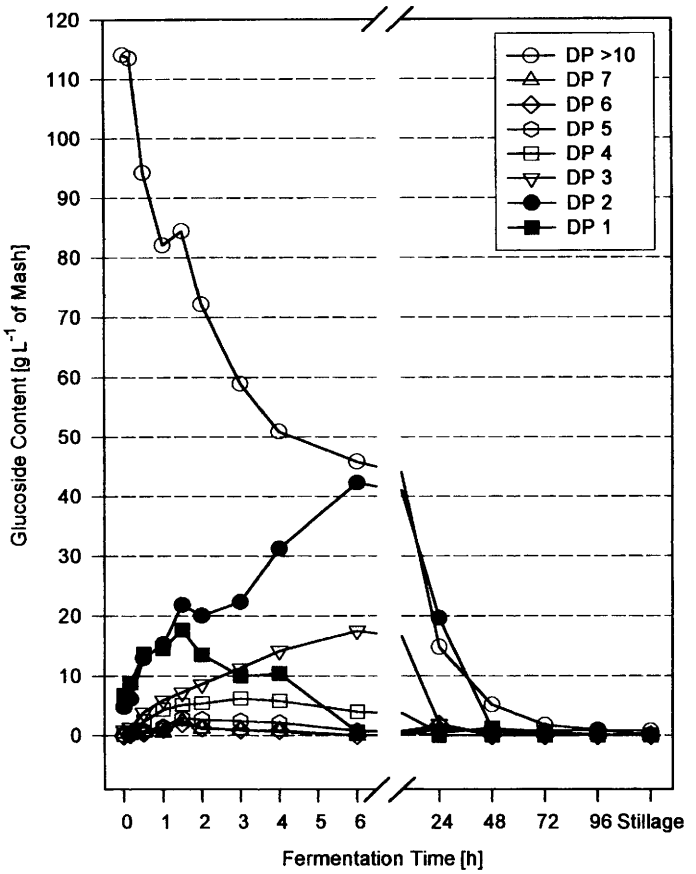

Fig. 6. Saccharification of corn mash using the enzyme combination OPTIMALT®. Yeast added after 30 min of saccharification; distillation after 96 h of fermentation.

of 30 min at a pH of 5.3–5.4. The best variety of wheat for use in autoamylolytic processes is "Alamo", with an AAQ > 95, independent of climate and growth conditions in different years of harvest.

3.2 Rye

Almost all varieties of rye show an AAQ > 95 (Aufhammer et al., 1993). The optimum conditions of the rye autoamylolytic enzyme system are the same as for wheat. But due to the content of pentosans, rye mashes often become very viscous. To avoid problems in mash treatment and fermentation, the viscosity of rye mashes should be reduced. This can be done either by using pentosanases, which are costly, or by using a certain time and temperature program (Sect. 6.2).

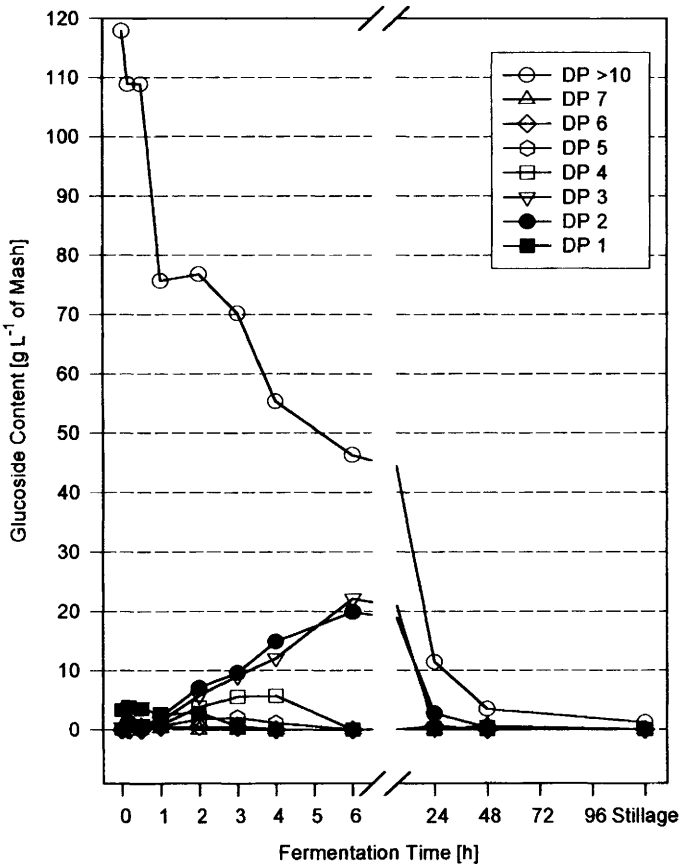

Fig. 7. Saccharification of corn mash using the enzyme combination OPTIMALT®. Yeast added when saccharification is started; distillation after 72 h of fermentation.

3.3 Triticale

The autoamylolytic enzyme system of triticale is maximally active at 55–60 °C and at a pH of 5.0–5.8 (Thomas et al., 1991; Senn et al., 1993). To reach a sufficient gelatinization of starch from triticale the triticale mashes must be heated to only 60 °C for 60 min after the starch has been completely released. The autoamylolytic system of triticale is stable at this temperature. A maximum ethanol yield is achieved at a mash temperature of 60 °C by adjusting the pH to 5.8 for 30 min and then lowering it to 5.2 for 30 min.

Starch degradation by autoamylolysis is very different from starch degradation by the action of technical enzyme preparations (Senn, 1995). In this work, degradation of starch in corn mashes was compared with autoamylolysis of starch in mashes from triticale (Fig. 8). After liquefaction, 1 L of corn mash

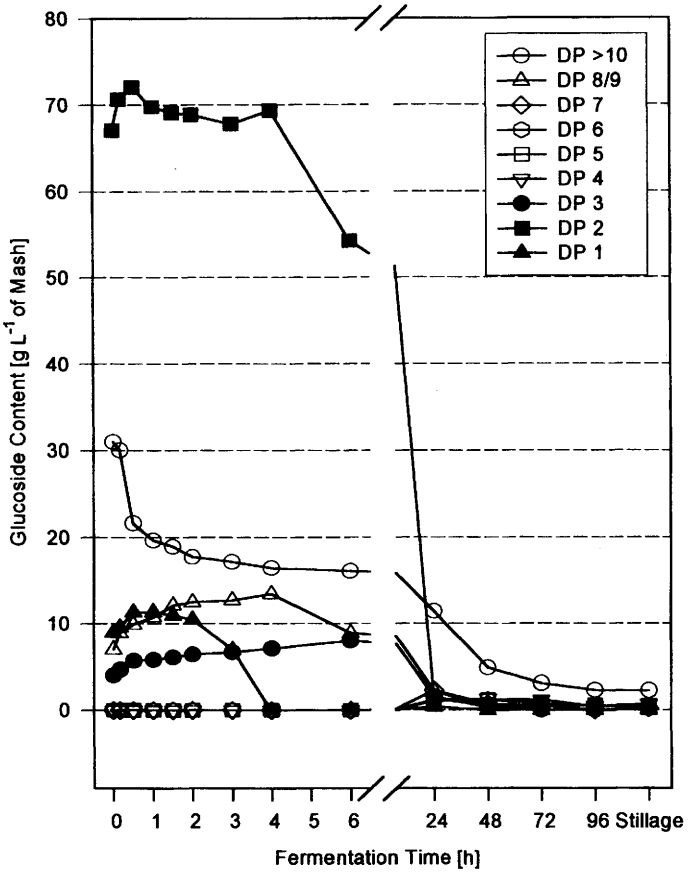

Fig. 8. Saccharification of triticale mash under autoamylolytical conditions. Yeast added after 30 min of saccharification; distillation after 96 h of fermentation.

contained about 110 g oligosaccharides in the DP > 10 fraction and about 5 g of directly fermentable sugars (maltose and glucose). In contrast, triticale mashes (autoamylolytically processed) had a DP > 10 content of 30 g L^{-1} mash and a directly fermentable sugar content of about 80 g L^{-1} mash. This fraction of directly fermentable sugars in corn mashes reached a maximum of about 40 g L^{-1} mash when saccharification was carried out with the enzyme combination OPTIMALT®.

The additional use of technical saccharification enzymes does not affect the process of starch degradation if mashes are processed under autoamylolytic pH and temperature conditions (Fig. 9). Autoamylolytic starch degradation leads only to small increases of the DP3 to DP7 fractions at the end of fermentation. But the addition of the α-amylase from *B. licheniformis* to the autoamylolytic process changes the situation and starch degradation is complete by the end of fermenta-

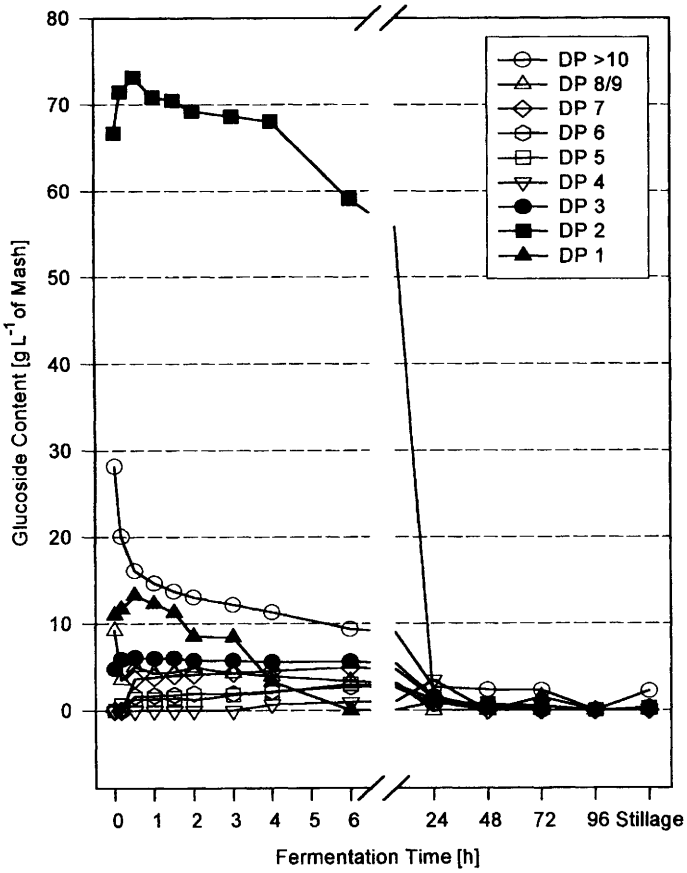

Fig. 9. Saccharification of triticale mash using the enzyme combination OPTIMALT®. Yeast added after 30 min of saccharification; distillation after 96 h of fermentation.

tion. This clearly shows the effectiveness of the autoamylolytic enzyme system. Further studies on the autoamylolytic properties of triticale which depend on growth conditions have been reported by Aufhammer et al. (1993, 1994).

4 Mashing Processes

4.1 Mashing Equipment

4.1.1 Wet Cleaning of Potatoes

Before processing potatoes, they must be cleaned and free from sand, stones, soil, and potato foliage. For this purpose washing rolls with a minimum length of about 3 m and a minimum diameter of 1.5 m are used. The potatoes pass

through the turning wash roll with countercurrent flow of warm water. After this, the potatoes pass through a stone catcher and are then delivered to an elevator which also has a countercurrent water flow. The elevator delivers the potatoes to the storage tank where they are weighed. Cleaning of the potatoes starts by washing them out from the potato storage cellar. From this washing channel, potatoes are pumped with a special centrifugal potato pump to the washing roll, which often requires the use of elevators. Water consumption in this washing process reaches 3–5 times the volume of the potatoes. Normally the water used in cooling down the mashes is used again for washing the potatoes, thereby minimizing the consumption of fresh water.

4.1.2 Grinding Raw Materials

One of the fundamentals of pressureless mashing processes is the thorough grinding of raw materials, which is usually done with hammer mills or dispersing machines and leads to a better digestion of starch.

4.1.2.1 Mills

For milling the raw materials usually only hammer mills are used in practice. These mills can be used under dry or wet conditions. When milling cereals under dry conditions, it is necessary to fit the mills wih a dust collector to avoid the settling of dust throughout the distillery. An advantage of dry processing is that milling can be done overnight, storing the meal in a hopper. To reach a sufficient degree of disintegration in the hammer mill a 1–1.5 mm screen is needed. Wet milling in-creases the throughput of raw materials but decreases the degree of disintegration. Therefore, water is added together with the material to be ground into the milling chamber.

An alternative to these two possibilities is to mill under dry conditions, simultaneously adding water to rinse out the meal, and then to pump it directly into the mash tub, using an eccentric screw pump situated below the mill. The rinsing water is delivered only to the meal chamber of the mill. Only the raw material is delivered to the milling chamber and, therefore, milling is carried out under dry conditions, while the formation of dust is completely avoided.

In practice, hammer mills are often fitted with 1.5 mm slot screens. These slot screens are more abrasion-resistant than perforated screens but are less efficient in milling. However, these slot screens are a good alternative when milling potatoes, since milling potatoes results in more abrasive wear than milling cereals due to the load of soil.

4.1.2.2 Dispersing Machines

The main purpose of disintegrating raw materials for alcohol production is to release starch from cell material. Therefore, ideally each single cell should be broken up, which is impossible to achieve using a mill. In ethanol production technology two kinds of rotor-stator dispersing machines are in use: in-line machines working in the continuously running Supramyl process (Misselhorn, 1980a) and batch machines from the ULTRA-TURRAX type used in the dispersing mash process (Fig. 10). The use of in-line machines ensures that all of the mash passes the dispersing head, but these machines are damaged by stones and sand delivered together with mash. If, in contrast, a mash tub is fitted with an ULTRA-TURRAX for batch processing, this makes the passing of mash through the dispersion head a statistical problem. Both systems lead to the same and very effective disintegration of raw materials; however, to drive one in-line machine, an electric motor power of 55 kW is needed in the Supramyl process, while working batchwise an electric motor power of only 17.5 kW

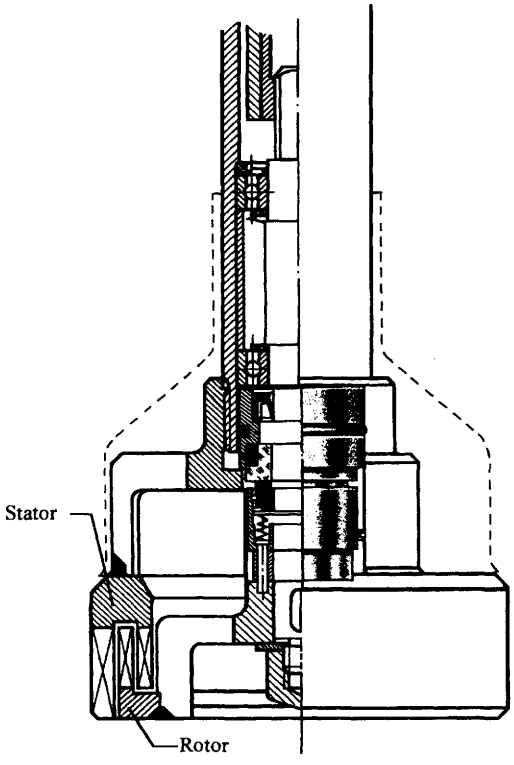

Fig. 10. Rotor-Stator dispersing machine (ULTRA-TURRAX type, IKA Maschinenbau, D-79219 Staufen).

is required for one of these machines. Thus, the investment needed for a batch machine is a third of that needed for an in-line machine.

This good effect of disintegration of raw material is possible, because the action of dispersing machines is not comparable with milling. There are four effects of disintegration that take place simultaneously and are overlapping:

- the mechanical shearing effect between the toothed rotors and stators,
- compression and decompression power, which leads to an intensive loosening of the structure of cell material,
- dispersion effect by splitting the mash stream into many single streams while passing the dispersion head,
- dispersion effect by microcavitation in very small regions with a high energy density.

By using an ULTRA-TURRAX in batch processing it is possible to process cereals and potatoes without previous grinding or steeping. And by generating high-frequency oscillations in the mash tub, a virtually complete release of starch is achieved and encrustations do not form in the mash tub.

4.1.3 Mash Tubs

A modern mash tub should comply with the following criteria:

- made from stainless steel,
- fitted with an effective and energy saving agitator,
- fitted with a sufficient cooling surface,
- easy and reliable cleaning and disinfection.

A mash tub should be cylindrical with a slightly domed bottom and top in order to achieve a good effect of agitation and to permit total discharging.

Most of the common mash tubs (Fig. 11) are fitted with impeller or propeller agitators. Pieper et al. (1990) showed that it is better to use a pitched-blade turbine for this purpose. The use of pitched-blade turbines saves about 50% of energy input for agitation of mashes compared to an impeller agitator. Furthermore these turbines guarantee an even distribution of whole cereal grains in the mash, starting a dispersing mash process, that allows, e.g., processing of cereals without previous grinding and steeping.

Agitators are commonly equipped with a drive from either the top or from the bottom. Selecting a drive from the top requires a drive shaft with a length corresponding to the height of the mash tub, and selecting a drive from the bottom in practice leads to problems with the seal.

For cooling mashes, the most common mash tubs are fitted with a cooling coil that is usually made of copper or stainless steel. To have a sufficient cool-

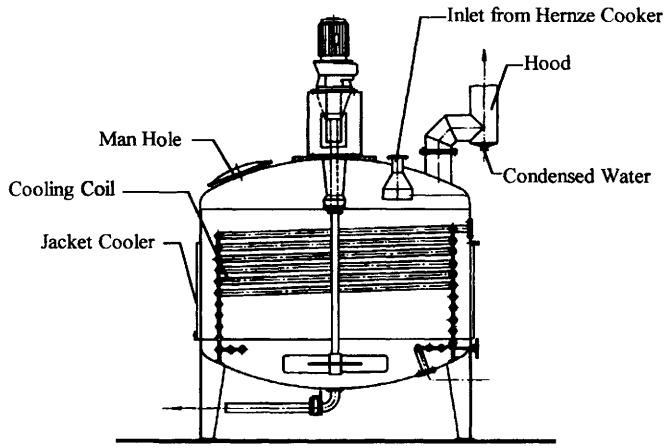

Fig. 11. Mash tub (Kreipe, 1981).

ing effect with cooling water at 10 °C, it is necessary to have a cooling coil of about 4 $m^2\ m^{-3}$ mash.

Cooling is not only affected by the cooling surface and water temperature, but also by the design of the cooling coil, which must permit a good circulation of mash around the whole coil in the mash tub. Therefore, a sufficient distance between the wall of mash tub and the cooling coil and between the single turns of the coil must be maintained. When designing a cooling coil, it must be ensured that there are no dirt-holding spaces around the spacers and fittings.

4.1.4 Heat Exchangers

In order to save energy, time, and cooling water some distilleries have started to use heat exchangers separated from the mash tub for heating and cooling mashes. Four types of heat exchangers are in use: spiral-plate heat exchangers, tubular heat exchangers, plate-type heat exchangers, and spiral-tubular heat exchangers.

In everyday practice spiral-plate heat exchangers have a strong tendency to form encrustations, both during the heating up of mashes before liquefaction and during the cooling down of mashes. This seems to be due to the low flow rates of mash between the spiral plates, which leads to sedimentation of mash solids and further lumping. Thus, the cross-sectional area gets smaller, and it is necessary to clean these spiral-plate heat exchangers regularly with water, using high flow rates to rinse out mash solid agglomerations, and afterwards with a hot sodium hydroxide solution (2% NaOH in water) to lower the risk of contamination which is ever present in distilleries.

The use of tubular heat exchangers is more successful. The tendency to form encrustations is quite low and they are quite easy to clean since cleaning balls can be pumped through the pipes. But in heating up cereal mashes there are problems too, due to the many 180° elbow fittings, which are design features of tubular heat exchangers. When cereal mashes, especially corn mashes, are heated up, gritty particles of the mash often show a tendency to sediment in the regions of the elbow fittings. Due to the degree of disintegration of the raw material pumped through the pipes, this sometimes results in clogging up the heat exchanger.

In the last few years, spiral-tubular heat exchangers have been installed in distilleries. Due to the design of these heat exchangers, in which spiral bent coils are welded to one another without elbow fittings, have the same advantages as tubular heat exchangers, but there is no problem treating cereal mashes. They are effective heat exchangers and they are easy to clean.

It is also possible to use plate-type heat exchangers in distilleries. But for heating and cooling cereal mashes these must be non-clogging plate-type heat exchangers. These are fitted with corrugated plates generating corrugated rectangular ducts. The single plates are kept apart only by the seals. Over the entire length of the plates there is the same slit width between the plates. This slit width sould be 10–12 mm to permit the use of these non-clogging plate-type heat exchangers in distilleries. It is very important that the mash flow rate is higher than 0.3 m s^{-1} to avoid sedimentation and agglomeration of mash solids inside the heat exchanger.

4.1.4.1 Processing with Heat Exchangers

Using heat exchangers in distilleries to heat up mashes, a rise in mash temperature to more than 55–60 °C inside the heat exchangers should be avoided. If gelatinization of starch takes place inside heat exchangers, the formation of encrustations can hardly be avoided, even if liquefaction enzymes are added.

If heat exchangers are installed in distilleries, they can be used in two different ways. After reaching a sufficient disintegration and liquefaction the mash can be pumped directly into the fermentation tanks, passing the heat exchanger, where it is cooled down to set temperature. In this case, saccharifying enzymes and yeast mash are added to the mash in the fermentation tanks.

Using the second method of processing, mash is first pumped through the heat exchanger and then back to the mash tub. During this circulation the mash is cooled down until the saccharification temperature is reached in the mash tub. If at this temperature saccharification enzymes are added, the mash is pumped to the heat exchanger and fermentation tank, where it is cooled down to set temperature. Yeast mash is also added after the mash has cooled.

4.1.5 Henze Cooker

Up to 30% of the distilleries in Germany are still working with a Henze cooker. The design of these pressure vessels is described in detail by Kreipe (1981) (Fig. 12).

The Henze cooker is the most popular pressure vessel used in European distilleries. It is manufactured from a cylindrical and a conical part with certain dimensions to achieve a virtually even distribution of vapor. The optimal dimensions are:

cylindrical height : conical height : cylindricaldiameter = 1 : 2 : 1.3

If the dimensions of Henze cookers are of these proportions, it is not necessary to install a stirrer if no milled raw materials are processed. Today, these pressure vessels should be made from stainless steel to avoid corrosion.

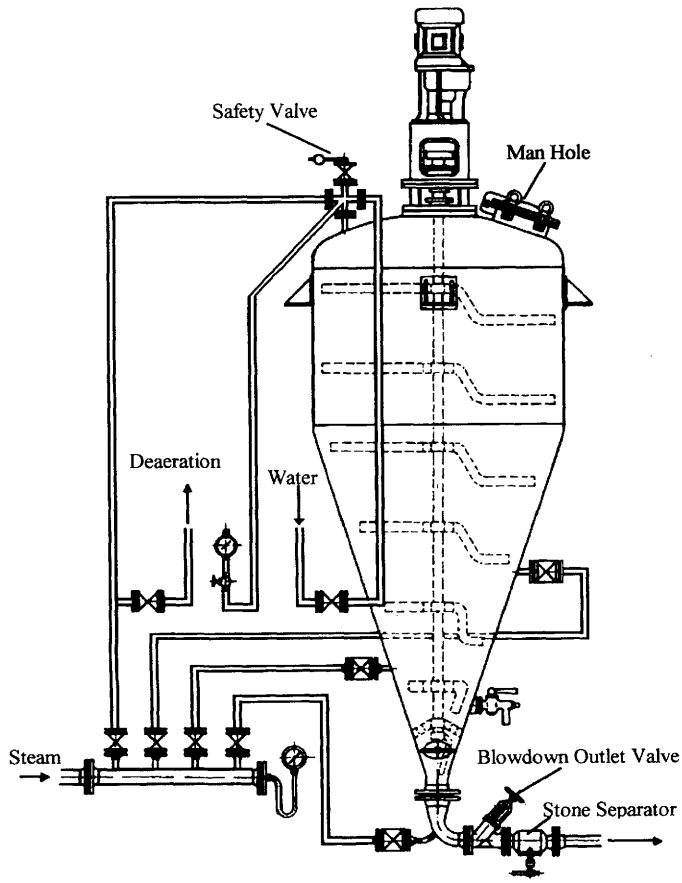

Fig. 12. Henze cooker (Kreipe, 1981).

The Henze cooker has to be fitted with a steam valve to inject live steam, and a blowdown outlet valve at the bottom of the cooker. About two steam inlet valves in the conical region and an additional deaeration valve at the top of the cooker are required. In addition, the cooker should be fitted with a manometer and a safety valve. The blowdown tube, leading mash from the cooker to the mash tub, should be sized to guarantee that the cooker is blown down within 20 min; otherwise intense caramelization takes place.

4.2 Pressure Boiling Processes

The term "Pressure Boiling Process" is applied to all processes in which the release of starch from raw materials and its gelatinization with water take place at temperatures above 100 °C. This process was developed before 1900 and is still in use today. It is commonly used for the processing of potatoes, corn, cassava, millet, and other starch-containing raw materials. Until 1975 it was virtually the only method used in Europe.

4.2.1 High Pressure Cooking Process (HPCP)

This method is widely used because of its applicability for almost all starchy raw materials. In general, there is no need for a size reduction of the raw materials. We find an almost complete release of starch from the raw materials which is gelatinized to a great extent. In addition, the use of high temperature and pressure is relatively safe in everyday operations.

The almost complete decomposition of raw materials in HPCP is achieved by the use of high temperature and pressure in the presence of water in a Henze cooker (Figs. 12 and 13). In this process step, whole grains or whole po-

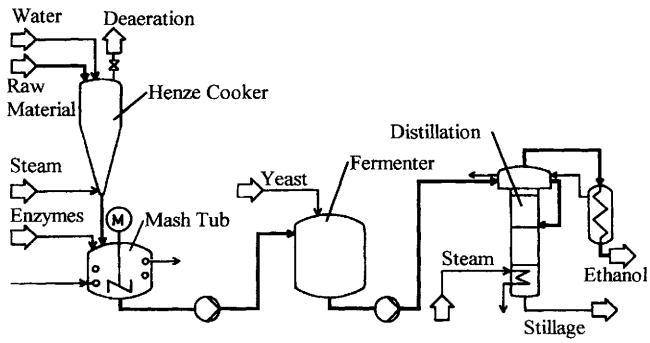

Fig. 13. High pressure cooking process (Pieper and Bohner, 1985).

tatoes are placed in the Henze cooker together with the required amount of water. Steam is introduced from the bottom of the Henze cooker to the material to be cooked, thereby raising the pressure slowly to an overpressure of 4.5–6 bar (140–160 °C), depending on the raw material used. The contents of the cooker are kept at this pressure for 40–60 min.

With this processing mode, pressure and temperature cause an extensive dissolution and gelatinization of starch in water, while preserving the outer shape of the grains. Once the raw material has been sufficiently cooked, the cooker contents is blown into the masher through a blow-off valve situated at the bottom of the Henze cooker. Only then does an extensive breakdown of the cell association and consequently the release of starch take place, due to the sudden pressure drop in the valve. Any remaining starch granules are exposed and immediatly gelatinized at this point.

The determination of the optimal time-point for blowing out the cooker contents is difficult. If the cooking process is stopped too early, the dissolution and gelatinization of the starch will be insufficient. This goes along with an inadequate penetration of the grains with water. As a result, the starch cannot be completely degraded during cooking, blowing out, and during further processing. This leads to ethanol losses and an increased risk of contamination during fermentation.

If, on the other hand, the cooking proceeds for too long, the result will be an increased formation of melanoidines and a severe caramelization of the mash due to the Maillard reaction. Consequently, losses of starch and sugars will occur. Furthermore, melanoidines inhibit the fermentation.

The mash is then cooled down in the mash tub and liquified by putting thermostable α-amylase from *B. licheniformis* into the masher before blowing out is started. Otherwise, bacterial α-amylase from *B. subtilis* can be added when a temperature of 75 °C is reached. For the reasons mentioned in Sect. 2.1, preferably α-amylase from *B. licheniformis* should be used in this processing mode.

Once the temperature of the mash has dropped to about 60–55 °C, glucoamylase from *A. niger*, which may be accompanied by fungal α-amylase from *A. oryzae*, is added for the saccharification of liquified starch. The mash is further cooled down to set temperature for fermentation. Yeast mash is usually used for the addition of yeast at 35 °C. The mash is pumped into a fermentation vessel and after 3 d of fermentation at 32–36 °C, the mash is distilled. The resulting stillage is used as feedstuff or as fertilizer.

The high energy consumption for HPCP, which amounts to about 7 MJ per lA for the mashing process only, led to the design of a process that could be pressureless and safe.

4.2.2 Bacteria-Free Fermentation Process of Verlinden (Verlinden Process, VP)

The Verlinden Process (VP) developed in 1901 in Belgium also uses a Henze cooker. In general, this process is only used for cereals. The pressure cooking conditions are the same as given for the HPCP (Sect. 4.2.1). But in this process mash tubs are not used. After the usual pressure treatment of mashes, they are blown out directly into special fermentation tanks fitted out with agitators. The vapor of this operation sterilizes the fermentation tanks. The mash which is agitated in the fermentation tank is cooled down by sprinkling the tanks. If microbial enzymes for liquefaction and saccharification are used, they are added to the mash at the usual temperatures. Dried distillers malt ore, a green malt slurry, must be disinfected with methanal (formaldehyde) before adding to the mashes.

Although this VP gives a certain guarantee that the fermentation runs free of contamination, the disadvantages of this process are obvious. The cooling rate is very low since the cooling surface is small, and sprinkling leads to the formation of hard scale. Furthermore, water consumption is very high and the process takes a long time. To start fermentation for the VP, pure culture yeast or fresh baker's yeast should be used in order to minimize the risk of contamination; however, this yeast is not ideally suited to the raw material that is processed. In addition, the fermentation equipment is expensive and the consumption of energy is high compared with other processes. Practical experience showed, that the VP does not yield more ethanol than the HPCP and is liable to the same risks of contamination.

4.3 Pressureless Breakdown of Starch

4.3.1 Infusion Processes

4.3.1.1 Milling and Mashing Process at Saccharification Temperature

Prior to 1950 this process was known as "Kaltmaisch-Verfahren" (KMV, cold mash process). It can be used to process wheat, rye, and triticale. This process is based on the enzymatic activities of used raw materials. These activities lead to dissolution and partial saccharification of starch without previous liquefaction (Kreipe, 1981). A precondition for the successful application of this process is that the raw materials are very finely ground (Kreipe, 1981). The milling of grains is usually carried out under dry conditions. The mill should be fitted with a dust collector, and the ground material is loaded into a storage hopper situated over the mash tub.

The thoroughly ground cereals (wheat, rye, triticale) are stirred into the mash tub which contains the necessary amount of cold water (Fig. 14). The water temperature must be below 15 °C, and agglomeration must be prevented. The mash is kept cold overnight and left to soak. The pH is adjusted to between 5.6 and 5.8. If microbial enzymes for starch liquefaction are used in the process, they can be added while stirring in the ground grain.

The next morning the soaked mash is heated to 50 °C by direct steam and kept at this temperature for about 30 min for a protein catabolic rest. Then the mash is heated to 58–60 °C, and this temperature is maintained for 60 min to provide a digestion, liquefaction, and saccharification rest. Afterwards the mash is cooled down to set temperature.

For liquefaction and saccharification distillers barley malt is normally added to the mash when heating up is started. If microbial enzymes are used for saccharification, they can also be added at this time, provided they are stable at 60 °C. At 35 °C, fermented yeast mash is added to the sweet mash. After 3 d of fermentation the mash is distilled and the stillage is used as feedstuff or fertilizer.

Some distilleries have started to use soaking water with higher temperatures in order to save steam consumption in the mashing process. The temperature may not exceed 50 °C, and soaking overnight is impossible because of the risk of infection. It is, however, recommended to allow a soaking rest of 1–2 h, otherwise the risk of contamination increases rapidly.

A disadvantage of this KMV process is that unfortunately the autoamylolytic enzyme system in cereal grains is not stable at 60 °C for more than 10–15 min. In addition, the enzyme activities vary greatly between different charges of raw material (Fig. 1), and with different charges of raw materials different particle-size distributions result in spite of the use of the same mill. Both of these factors lead to an incomplete release and degradation of starch during the mashing processes. This poor breakdown of starch and the low maximum temperature used in this process give rise to an increased risk of contamination during fermentation. Therefore, this process is rarely used now.

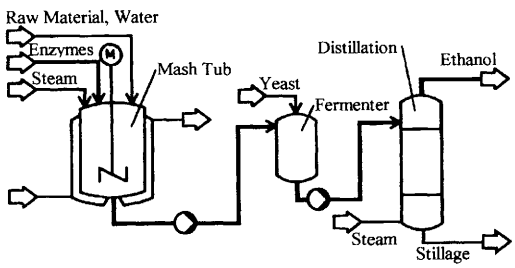

Fig. 14. Milling and mashing process, "cold mash process (Kaltmaischverfahren)" (Pieper and Bohner, 1985).

4.3.1.2 Große-Lohmann-Spradau (GLS) Process

This process was developed to reduce energy consumption in distilleries using pressure boiling processes. In these distilleries, the old Henze cooker is often used as the first of two mash tubs. This GLS process is mostly used to process potatoes, or sometimes corn (Fig. 15).

To run this process a very efficient pump, usually a centrifugal pump, is installed between mash tub 1 and mash tub 2. This pump permits the pumping of high amounts of mash from the bottom of mash tub 1 (normally an old Henze cooker) back to the top of mash tub 1. A second pipe allows pumping of the mash into mash tub 2.

To start the GLS process, raw materials are milled by a hammer mill. In these mills, slot sieves with a slot width of about 1–1.5 mm are often used. The use of perforated metal screens with a clear space of 1.5–2 mm in these hammer mills leads to better results in fineness of grinding. Milling can be done under dry or wet conditions. For grinding under wet conditions, water is poured into the grinding chamber together with the raw materials. This kind of milling leads to a faster but less efficient grinding compared to dry conditions. Using dry conditions, water is used only to rinse out the ground material from the mill.

To start the GLS process, water with a maximum temperature of 50 °C is filled into mash tub 1 and liquefying enzymes are added. Then the circulating pump is started, and the water is pumped from the bottom of mash tub 1 back to the top of it. After grinding, the mash is also pumped into mash tub 1. The mash flows are brought together directly over mash tub 1 and pumped over a mixing plate installed in the head area of this vessel. The mash is heated with live steam to 90 °C while both pumps are running. When mash tub 1 is filled up to its maximum level, the milling and pumps are stopped and the temperature is maintained at 90 °C for 1 h. After this liquefaction rest, the mash is pumped into mash tub 2 and cooled down. When a temperature of about 55 °C is reached, saccharifying enzymes are added and a saccharifying rest of 15–30 min may be carried out. Afterwards, cooling is continued and at 35 °C the yeast mash is added.

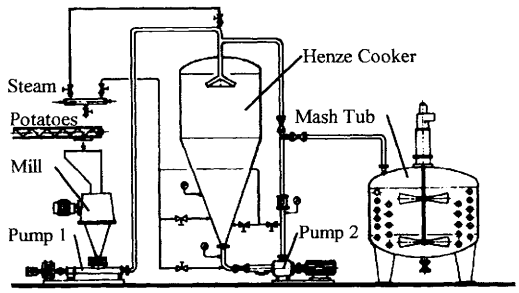

Fig. 15. Mashing process according to Große-Lohmann-Spradau (Kreipe, 1982).

This GLS process permits good processing of potatoes. The result of the process, however, depends strongly on the milling efficiency, especially for processing corn. It is impossible to reach a total release of starch from plant cells with a reasonable expenditure of energy by using mills. Undigested starch leads to problems mainly during fermentation because of higher viscosities and risks of contamination.

4.3.1.3 Milling and Mashing Process at Higher Temperatures (MMP)

The milling and mashing process at higher temperatures (MMP) is often used in distilleries that have invested in new mashing equipment since 1980. It permits the processing of all starchy raw materials. The flowsheet of this process is shown in Fig. 14.

At the start of MMP, the starchy raw materials are usually ground in a hammer mill normally fitted with a 1.5 mm screen and a dosing pump for liquefaction enzymes. Cereals are mostly milled under dry conditions, using water to rinse out ground material. Liquefaction enzymes are added together with rinsing water which may have a temperature of up to 55 °C to reduce energy consumption. For milling potatoes, the amount of rinsing water used in the mill is very low, while the ratio of cereals to be ground to water has to be at least 1:1. Mash is delivered from the bottom outlet of the mill to the mash tub with an eccentric screw pump.

In the mash tub, after pH adjustment to 6.0–6.2, the mash is heated to liquefaction temperature with live steam. Heating can also be done in the delivery line using a temperature-controlled steam injector. The liquefaction temperature depends on the raw material (potatoes: 90–95 °C; maize: 80–90 °C; wheat, rye, triticale: 65–70 °C). After a liquefaction rest of about 30 min, the mash is cooled down to saccharification temperature, and saccharification enzymes are added. The pH should be adjusted to 5.3–5.5 using concentrated sulfuric acid to optimize the activity of the enzymes added to the mash. Afterwards the mash is cooled down to set temperature, and usually yeast mash is pumped into the mash.

Sometimes, to save energy, the hot stillage coming out of the distillation column is used to heat up the mash by directing it through the cooling coil located in the mash tub. It is also possible to heat up potato mashes in the delivery line with hot stillage between the hammer mill and the mash tub using tubular heat exchangers or spiral-plate heat exchangers. But the temperature of the mash may not exceed 55 °C to prevent gelatinization of starch. Although liquefying enzymes are added to the mash, encrustations can not be avoided if the gelatinization temperature is exceeded in the heat exchanger. In practice, spiral-plate heat exchangers in particular have a tendency to build up encrustations. Since the temperature may not exceed 55 °C in a heat exchanger, it

should not be employed while processing cereals; this temperature can easily be reached using the warmed up cooling water for rinsing out the ground material from the mill.

The MMP depends entirely on the effect of milling and the efficiency of the enzymes used, especially for saccharification. Incomplete release and degradation of starch in the process followed by increasing risks of contamination and losses in ethanol yield may result if milling and enzyme action are not optimal.

4.3.2 Recycling Processes

4.3.2.1 Stillage Recycling Process (SRP)

In the endeavor to reduce energy consumption in pressureless processes, Pieper and Jung (1982) developed the stillage recycling process (SRP). This process allows optimum utilization of thermal energy contained in the stillage, but this process may only be used with wheat, rye or triticale. The flow sheet of the SRP is shown in Fig. 16.

The recycling procedure of the stillage is important for the SRP. The ethanol-free stillage coming out from the continuous mash still, with a temperature of about 102 °C, is delivered into an intermediate vessel. Driven by the hydrostatic head, the stillage is led to a centrifugal decanter for separation of the solids (seed shells, germs, endosperm residues) from the liquid fraction. The stillage is separated into two phases: a solid phase, containing about 30% DS, and a liquid phase with a low solids concentration. The solid phase is used as feedstuff, and the liquid phase is directly recycled in the mashing process to substitute for needed process water. The residual 20–30% of the liquid phase which is not needed in the process is also used as feedstuff. The liquid phase of stillage

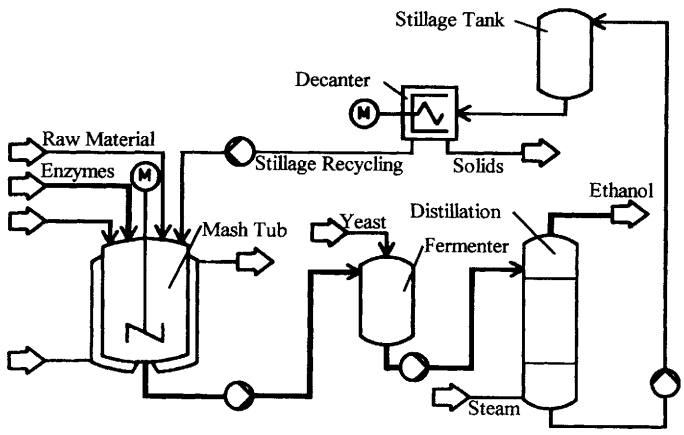

Fig. 16. Stillage recycling process (Pieper and Bohner, 1985).

is now used as process liquid in the following mashing process. The mash is fermented, and with distilling of this mash the stillage cycle is closed.

To run the SRP, the liquid phase of stillage obtained from the centrifugal decanter is pumped into the mash tub. While agitating, the pH is adjusted to 6.3–6.4 using technical calcium hydroxide. The temperature of stillage in the mash tub reaches 80–85 °C if the process is started in this manner.

One half of the thoroughly fine milled raw material is loaded into the mash tub while the agitator is running. Because of the high temperature of the stillage, charging must be done slowly and evenly to avoid agglomeration. This is necessary since the high temperature results in a rapid gelatinization of starch charged to the mash. Just after beginning to load raw material into the mash tub, thermostable α-amylase from *B. licheniformis* is added to the mash. This prevents an unwanted increase in viscosity. Upon adding the raw material to the mash, the temperature slowly decreases and the efficiency of the thermostable α-amylase slowly diminishes. Therefore, when 50% of the raw material has been added, charging is stopped and, if necessary, the mash is cooled down to 74 °C. At this temperature bacterial α-amylase from *B. subtilis* is added, and the second half of the raw material is charged. The mash normally has a temperature of 70 °C and a pH of 6.0 if loading of raw material is done properly.

The mash is then cooled down to 57 °C and the pH is adjusted to 5.5. After that distillers' dried malt immediately is added. Upon reaching 55 °C, glucoamylase from *A. niger* is added to the mash. After further lowering the pH to 5.0, fungal α-amylase from *A. oryzae* is added. A temperature of 55 °C is maintained for 1 h as a saccharification rest. Following the saccharification rest, the mash is cooled down to set temperature and yeast mash is added. The mashes produced with the SRP can be completely fermented within 44 h.

4.3.2.2 Dispersing Mash Process Developed at Hohenheim University (DMP)

The dispersing mash process (DMP) with stillage recycling as one of its characteristic components, was developed by Pieper and Senn (1987). The flowsheet depicting the process is shown in Fig. 17.

The DMP allows processing of all starchy raw materials, as well as Jerusalem artichokes and sugar beet. It is characterized by three important features:

(1) decomposition of raw materials employing a rotor-stator dispersion machine;
(2) use of stillage recycling to reduce energy consumption and to optimize fermentation efficiency;
(3) use of OPTIMALT®, an optimum enzyme combination for saccharification of mashes from pressureless processes; this also leads to an optimum decantation of stillage.

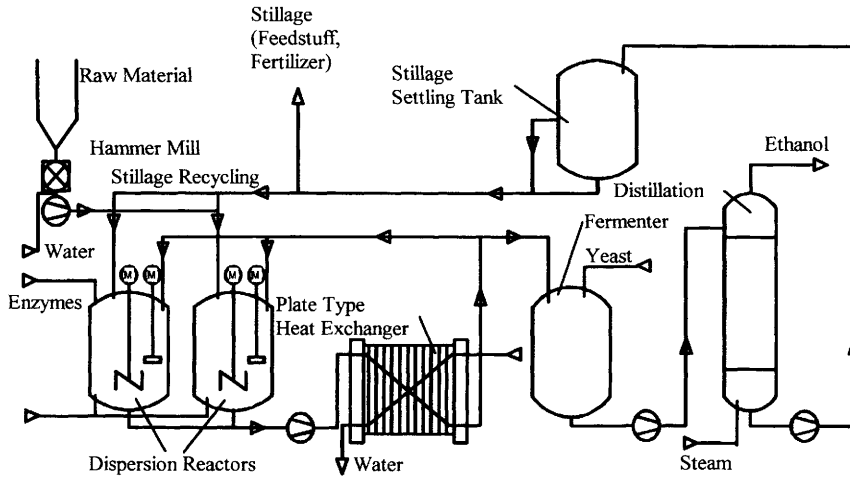

Fig. 17. Dispersing mash process.

The DMP permits processing of cereals and potatoes without milling or after just a coarse milling. If there are more than one or two mashes processed per day in one mash tub, it is recommended to use a hammer mill fitted with a 4 mm screen to grind the material. This reduces the time used for releasing starch from the raw material.

The stillage used for stillage recycling must be separated from the solids contained in the stillage. This can easily be done by employing a separation tank. The stillage from the distillation column is delivered into this separation tank for storage. After about 3–5 h, the stillage is sedimented and clearly consists of two phases. The solid-free phase is used in the process again. It amounts to 50% (wheat, rye, triticale) and to 70% (corn) of the total stillage. The solids containing phase of stillage, which is barely a fluid, is used as feedstuff or as fertilizer.

When processing potatoes, the unseparated original stillage is used for recycling in an amount of about 15% of total stillage.

At the start of the DMP, the necessary amount of solid-free stillage is pumped into the dispersing reactor, which is used as mash tub. The pH is adjusted using hydrated lime (calcium hydroxide) depending on the properties of the liquefying enzymes used.

The coarse-milled raw material is pumped into the dispersion reactor using as little water as possible for pumping, which should have a maximum temperature of 60 °C. At the start of the process, the temperature of the mash is maintained at liquefaction temperature by injecting live steam. The rotor-stator dispersion machine is turned on when charging is completed and the mash is treated until a sufficient release of starch is guaranteed. The degree of disintegration is determined using a special hydrosizer (Sect. 13.3.1). When the hydro-

sizer shows that sufficient degree of disintegration, dispersing is stopped and the mash is cooled to saccharification temperature by using either a special plate-type heat exchanger outside or a cooling coil inside the dispersion reactor. For saccharification at 53 °C OPTIMALT® is added to the mash. After a short saccharification rest, which is not necessarily required, the mash is directly pumped into the fermentation tank, passing the plate-type heat exchanger where the mash is cooled down to set temperature. Yeast mash is admixed when the mash stream has passed the cooler. If a cooled coil is in use, the mash is cooled down to set temperature inside the dispersion reactor and yeast mash is added.

Several dispersion reactors can be loaded in succession with mash, dispersed and cooled. This multi-batch processing leads to a quasi-continuous processing which, however, allows the individual determination of the degree of disintegration for every single mash. The dispersion reactor may have a volume of up to 15 m^3 to process up to 3 t of cereals for instance in one mashing.

The DMP leads to a virtually total release of starch from plant cells. In addition, the use of OPTIMALT® for saccharification results in a complete degradation of starch, high ethanol yields, and good settling properties of the stillage. The use of stillage recycling guarantees a rapid start of the fermentation, a reduced length of the fermentation, and optimum conditions for yeast propagation. Hence, DMP is an environmentally acceptable and energy saving process that yields up to 66 lA per 100 kg starch in industrial plants.

5 Processing Potatoes

Potatoes used in alcohol production should be in good condition and free from plant diseases. This is a problem, since industrial potatoes are harvested only from September until the end of October, and it is impossible to process all the harvested potatoes in that time. Therefore, it is necessary to store potatoes before processing, for which special store houses are in use. They must be fitted with aeration and washing channels. In one of these store houses with, e.g., a length of 31 m and a width of 15 m, $2 \cdot 10^3$ t of potatoes can be stored with a bed depth of 6.5 m. It is possible to store potatoes up to a bed depth of about 12 m. To maintain potatoes in good condition during storage, it is necessary to keep them cool in the storage houses. Therefore, air is blown into the aeration channels below the bed of potatoes. Cold air is passed through the bed of potatoes from the bottom to the top of the storage house and leaves through air registers on top of the roof. In autumn this aeration is done at night to use as cool air as possible. Potatoes should be kept at temperatures between +4 °C and +7 °C. During the loading period aeration of the storing houses is also necessary to avoid formation of condensed water on the surface of cold potatoes (Kreipe, 1981).

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Before processing potatoes they must be washed thoroughly, as described in Sect. 4.1.1, to minimize the risk of contamination with, e.g., spores of a *Clostridium* sp. contained in soil adhering to potatoes. Growth of these microbes in mashes may result in the formation of propenal (acrolein) during fermentation.

If the HPCP is used to process potatoes, washed potatoes are loaded into the Henze cooker. They are first steamed from the top of the Henze cooker using direct steam, while condensate draining off at the bottom. After about 15 min, when the cone of the Henze cooker is warmed up, steaming from the top is stopped and steaming from the bottom is started. This is done by closing the condensate outlet valve and opening the air outlet valve a little at the top of the Henze cooker. In this way the pressure is increased to 5 bar in 30–40 min. The potatoes are kept at this pressure for about 20–25 min, and then the content of the Henze cooker is blown out into the mash tub. It is possible to adapt this steaming process to different kinds of potatoes by varying the time the potatoes are kept at maximum pressure.

In pressureless processes, washed potatoes first have to be ground thoroughly. For thispurpose hammer mills can be used; however,employing a rotor-stator dispersion mashine results in a better degree of disintegration.Using DMP it is also possible to process potatoes in a mash tub without previous grinding. Hence, potatoes can be loaded into the mash tub at a rate of about 10 t h^{-1}, the same rate that can be reached by milling potatoes. A sufficient degree of disintegration is reached if by mash hydrosizing (Sect. 13.3.1) the coarse fraction is <1 mL and the sum of coarse and suspended fractions is <1.5 mL. Since BAB (see Sect. 2.1.3) does not work in potato mashes, the pH has to be adjusted to 6.0–6.2 for liquefaction. To complete the gelatinization of potato starch, the mash temperature must reach at least 80–85 °C. But heating up potato mashes to 92–94 °C lowers the viscosity of mashes and cooling is accelerated. Low viscosities also accelerate the fermentation and reduce the risk of contamination. A further acceleration of fermentation can be achieved by using recycled stillage in the process. In mashing 7.5 t of potatoes, 2 m^3 of original stillage, draining from the still, can be used.

6 Processing Grain

If HPCP is used for processing grain it is important to load enough water into the Henze cooker before starting the steaming process. Normally, therefore, about 300 L water are used per 100 kg of grain of any variety. The required amount of water is pumped into the Henze cooker, and the steaming valve at the bottom is opened a litte to suspend the grain. Then the grain is loaded into the Henze cooker and after closing, the bottom steaming valve is opened. The grain has to be evenly distributed throughout the whole volume as it is very im-

portant that all the single starch granules come into contact with a sufficient amount of hot water to reach a complete gelatinization. Therefore, the side steam valves should also be opened.

Steaming should take about 40–45 min to raise the pressure up to 5 bar. The content of the cooker should be kept for about 40–45 min at this pressure. The time needed to hold different grains at maximum pressure can vary from 25 to 60 min. Therefore, the progress in steaming should be examined by taking a mash sample from the sample outlet at the bottom of the Henze cooker. This sample is poured into a sieve and is visually examined. The seed coats should be completely separated from endosperm fragments before the cooker is blown out during about 20 min.

The Henze cooker should be fitted with an agitator for processing milled grain products with HPCP. The agitator is necessary to obtain a good suspension of the raw material in water. The water temperature may not exceed 50 °C. Otherwise, the milled product becomes lumpy and gelatinization and solubilization of starch remain incomplete. The time needed to keep ground material at maximum pressure can be reduced to 10–20 min.

It is necessary to use different conditions in processing different varieties of grain in pressureless processes. Hammer mills or dispersion machines are used when milling grain for alcohol production.

Milling of grain for alcohol production is normally carried out with hammer mills fitted with a 1.5 mm screen. The degree of disintegration reached by this method is sufficient for ethanol yields of up to 62 lA per 100 kg FS if processed grain shows high autoamylolytical activity. If a sufficient autoamylolytical activity is not present in the grain to be processed – and in practice this is the normal case – the losses in ethanol yield increase. The mill has to be fitted with a 1.0 mm or 0.5 mm screen to reach an almost total digestion of starch and ethanol yields of about 64–65 lA per 100 kg FS during mashing, especially if the grain or corn is waxy or dried after harvesting at high temperatures, inactivating the autoamylolytical enzyme system. But this leads to a high energy consumption and a low throughput in milling, and is, therefore, not economical. Furthermore, it is impossible to grind corn with 0.5 mm screen, due to the fat content of corn which leads to the clogging of screens.

If dispersion machines are used for the digestion of grain, the grain is first coarse milled using a hammer mill fitted with a 4 mm screen. The coarse ground material is pumped into the mash tub and dispersed there. A sufficient degree of disintegration is reached if by hydrosizing, the ratio between the coarse and the sum of the coarse and suspended fractions is <1 mL:3 mL. This guarantees ethanol yields of 64–65 lA per 100 kg FS, if no further mistakes are made during mashing and the fermentation process. Using DMP the mash is dispersed after the mash tub is filled up. Then the mash is heated up, and while dispersing it is kept at liquefaction temperature until disintegration is completed. If only one or two mashes are processed per day, grain can be

loaded directly into the mash tub without previous grinding. But dispersing the mashes takes twice the time of that required with coarse ground material; otherwise, it is not necessary to invest in a mill. This is quite important too, since it needs no daily cleaning of the hammer mill.

The use of BAB for liquefaction of mashes is recommended using stillage recycling in grain processing. Liquefaction of grain mashes can thereby be carried out at a pH of 5.0, making it possible to run the whole mashing process at this single pH value.

6.1 Wheat

To complete gelatinization of wheat starch, a temperature of about 65 °C is required. If, due to contamination of the raw material, there is a great risk of contamination in mashes, the liquefaction temperature may be raised to 75 °C. Higher mash temperatures lead to a better pasteurization effect, but results in losses of ethanol of about 2–3 lA per 100 kg FS. A protein catabolic rest during the increase of the mash temperature is not required. A maximum temperature of 65 °C has to be kept for at least 30 min to reach a sufficient liquefaction. During cooling of wheat mashes, a saccharification rest is not required, but it leads to a quick start of fermentation. A saccharification rest may be kept at 52–55 °C for about 15 min.

6.2 Rye

The processing of rye is affected by the content of pentosans. A high content of pentosans leads to high viscosities in mashes which become evident at the beginning of the process, when ground rye is solubilized in water. These high viscosities persist during the mashing process and the fermentation. This problem can be solved by using pentosanases, which are rather expensive, or by running a certain mashing program (Quadt, 1994; Ingledew et al., 1999; Wang et al., 1997). If pentosanases are employed in the process, they are added to the mash together with liquefying enzymes; the liquefaction temperature may not exceed 60 °C, since pentosanases are not stable at temperatures higher than 60 °C.

The optimum temperature for liquefaction of rye mashes is 60 °C (Quadt, 1994). Temperatures of more than 70 °C result in losses of ethanol yields of up to 3 lA per 100 kg FS. To process rye without using pentosanases, a pentosan catabolic rest at the beginning of process is required. This catabolic rest has to be carried out at 50 °C and a pH of 5.0. Therefore, a needed amount of stillage and/or water is filled into the mash tub to reach a temperature of about 50 °C. If only stillage is used, it must be cooled down. Then the pH is adjusted to 4.6–4.8, the milling of rye is started using a 4 mm screen, and the ground material is

pumped into the mash tub using warm water. If some ground rye is pumped into the mash tub, BAB is added to the mash as a liquefying enzyme. The temperature of 50 °C and a pH of 5.0 is maintained while filling up the mash tub and during further dispersion of the mash. This pentosan catabolic rest should be kept for 30 min to reach a sufficient degradation of pentosans by the action of enzymes contained in rye. The mash is then heated to 60 °C for liquefaction while disintegration of rye is completed by dispersing. Liquefaction at 60 °C should be carried out for at least 30 min or until disintegration is completed, if the latter lasts longer. After that the mash is treated as usual; with this mashing program a further addition of pentosanases only shows marginal effects.

This mashing program described above can also be used if only milling is used for disintegration. However, milling rye with a 1.5 mm screen results in lower degrees of disintegration followed by losses in ethanol yields of about 3 lA per 100 kg FS.

6.3 Triticale

The way to process triticale is the same as described for wheat (Sect. 6.1), because there is no problem with pentosans in mashing triticale. Triticale is a very important raw material for distilleries, due to the high autoamylolytical activities in some varieties. How to make use of these effects is shown in Sect. 8.

6.4 Corn

6.4.1 Dried Storable Corn Grain

Processing corn for ethanol production is influenced by the high temperature needed for gelatinization of corn starch and the presence of varying amounts of horny endosperm in the corn. The horny endosperm of corn is elastic. This leads to problems in milling since milled corn remains gritty, even if a 1.5 mm screen is used. The use of smaller screens is not recommended due to the fat content of corn, which leads to very small throughputs in milling or to clogging of screens. Gritty particles obtained from milling will not completely dissolve during mashing and fermentation. This leads to slow fermentations and losses in ethanol yields. A virtually complete release of starch is only guaranteed using HPCP or DMP. However, it is impossible to avoid ethanol losses using HPCP, due to the Maillard reaction and caramelization during the steaming process.

To liquefy corn mashes, it is necessary to heat them to 80 °–85 °C for at least about 30 min. Up to 90% of the water needed can be substituted by stillage if the DMP is used. If stillage recycling is carried out in the process, it is recommended to add BAB to liquefy the mashes at a pH of 5.0. After loading the stil-

lage and/or hot water obtained from cooling facilities into the dispersing mash tub, coarsely ground corn is pumped into the mash tub. If only one or two mashes are processed a day, it is possible to load corn into the mash tub without previous grinding. In either case, the mash is immediately heated up to 80 °C and dispersed at this temperature until the mash hydrosizer shows sufficient results (Sect. 13.3.1). Then the mash is treated as usual. The fat content of corn, disadvantageous in milling, is an advantage in fermentation. There is no foaming, and the fermentation tanks can be filled up almost completely.

6.4.2 Corn Grain Silage

Corn grain silage may be processed with the HPCP. A maximum pressure of 5 bar in the steaming process has to be maintained for about 20 min under the conditions described above. In either case, using HPCP or pressureless processes, the amount of water in the mashing process requires correction for the water content in corn grain silage. The ratio of starch to water in the process should be about 1:6.

When processing corn grain silage with pressureless processes, the raw material must be ground either by milling or dispersing. If hammer mills are used, the problems which arise are the same as those mentioned in Sect. 6.4.1. The way to process corn grain silage using DMP is also the same as for storable dried corn grain. When working with a pressureless process, it only has to be taken into consideration, that the pH value has to be observed when loading the corn silage into the mash tubs. Lactic acid, contained in the silage, leads to an acceleration of the fermentation. By using stillage recycling in the process, it is possible to reduce the fermentation period to only 2 d. This is impossible when processing dried corn grain.

Ethanol yields with corn grain silage amount to about 61 lA per 100 kg starch using HPCP (Pieper and Pönitz, 1973) and about 63 lA per 100 kg starch when working with pressureless infusion processes (Treu, 1991). Using DMP for processing corn grain silage, ethanol yields amount to more than 64 lA per 100 kg FS. However, ethanol yields reported in the literature which are not related to FS must be regarded with caution (Sect. 13.2). That is especially true for corn grain silage, which contains mono- and disaccharides, and lactic acid – substances leading to errors in other analytical methods.

6.5 Barley

The husks of barley cause some problems in processing barley with pressureless processes. Using screens smaller than 4 mm in milling, the throughput is greatly reduced. A further problem is the formation of pearl barley in milling,

even if 1.5 mm screens are used. Pearl barley swells strongly during the mashing process, and the starch contained in it can not be released. Therefore, a dispersion step is essential in processing barley. A third problem in mashing is the β-glucan content in barley. β-Glucan leads to high viscosities in barley mashes from the beginning of the mashing process. If the mashes are heated up to starch gelatinizing temperature, viscosity increases and cannot be controlled. The breakdown of β-glucan can be carried out by using β-glucanases, which are quite expensive, or by using a certain mashing and temperature program thereby utilizing β-glucanases contained in the barley.

It is impossible to process barley, even using DMP, without previous grinding (Heil et al., 1994). This is done with a 4 mm screen in a hammer mill. The necessary amount of water and/or stillage is loaded into the mash tub and adjusted to a pH of 5.2 and a temperature of 40–50 °C. For liquefaction, the addition of BAB to barley mashes is recommended. Then ground barley from the hammer mill is pumped into the mash tub and dispersion is started. To break down β-glucan, the mash has to be maintained at the aforementioned pH and temperature conditions for about 30 min. After that the mash is heated to a liquefaction temperature of 80 °C and kept at this temperature for at least 30 min. When the result of mash hydrosizing is satisfactory (Sect. 13.3.1) the dispersion machine is stopped and the barley mashes are treated as usual.

7 Processing Tropical Raw Materials

7.1 Sweet Sorghum

Sweet sorghum is a raw material containing fermentable sugars. Since it does not keep well after harvest, it cannot be imported from tropical countries. There have been some attempts to grow sweet sorghum in Austria and Germany (Diedrich et al., 1993; Salzbrunn, 1982). To obtain ethanol from sweet sorghum it is first necessary to produce a sugar juice from the plant stems. This can be done in two ways: The first possibility is water extraction with a countercurrent extraction plant; the second way is to press the sugar juice from the plant stems using a roller mill. In both cases it was possible to recover about 91–95% of total sugar contained in the plants.

Fermentation caused no problem, but it was necessary to pasteurize the sugar juice to avoid contaminations. Batch fermentation was finished within 40 h. An ethanol yield of about 58 lA per 100 kg of sugar can be expected (Salzbrunn, 1982).

The sugar juice obtained by extraction or pressing does not keep well. Therefore, it is necessary to concentrate the juice up to 80° Bx or 74% DS using a downflow evaporator. After chilling the sirup is extremely viscous, or even solidifies and is storable.

7.2 Sorghum Grain

Sorghum grain should only be processed using HPCP because of the waxy structure of the starchy endosperm (Kreipe, 1981). However, the experience with corn using pressureless processes, especially DMP, demonstrates that it is possible to process waxy raw materials with pressureless processes very effectively. There is no doubt that it should be possible to process sorghum grain the same way as corn. Using DMP and stillage recycling, ethanol production from sorghum grain saves much energy and is environmentally friendly.

7.3 Manioc

Manioc products should also be processed by the HPCP (Kreipe, 1981). About 350 L of water are needed for 100 kg of manioc in the cooking process. The pressure cooking conditions have been given as 3.5–4 bar for about 30 min. Saccharification has to be carried out using bacterial amylases, since saccharification using malt remains incomplete. During pressure cooking it is possible to blow out hydrocyanic acid using the deaeration valve.

Worldwide more and more varieties of manioc are cultivated which do not contain cyanogenic glucosides. Therefore, it should be possible to use pressureless processes. Furthermore, it should also be possible to blow out hydrocyanic acid by keeping manioc mashes at boiling temperature for about 10–20 min; the cyanogenic glucosides are not stable at this temperature. This could be a way to process manioc with more energy savings.

8 Mashing Processes Using Autoamylolytical Activities in Raw Materials

As shown in Sect. 6, it is necessary to heat cereal mashes up to temperatures, some degrees higher than gelatinization temperature in order to reach a complete gelatinization of cereal starch. However, the autoamylolytical enzyme system is not stable at these temperatures. Therefore, if the autoamylolytical enzyme system is to be used in mashing processes, a way must be found to complete gelatinization of starch without inactivating the enzyme system. This can be achieved by using a certain time, temperature, and pH combination during mashing processes (Rau et al., 1993; Senn et al., 1991). But one has to take into consideration, that this mashing program differs with different raw materials and combinations of raw materials.

Processing under autoamylolytical conditions is more labor intensive than other processes. The conditions (temperature, pH, and time) for an autoamylo-

lytical process, wich are described in the following sections, have to be followed exactly, and the cleaning procedures in the distillery are very important. This is due to low maximum temperatures that are maintained only for minutes. But using stillage recycling and taking care of the yeast cultivation guarantees a rapid start of the fermentation and the best chance to avoid contamination.

Besides all this, a only 20% of the normal amount of saccharifying enzymes used assures processing under disadvantageous conditions.

8.1 Processing Wheat

It is possible to process wheat with an AAQ of 95% or higher without the addition of enzymes. It is necessary to grind the material thoroughly or to use a hammer mill fitted with a 4 mm screen, combined with further use of the dispersing mash process. In either case, water and/or stillage is pumped into the mash tub, and after adjusting the pH to between 5.2 and 5.5, wheat is ground in the hammer mill and pumped into the mash tub; BAB is then added as liquefying enzyme. The mash temperature in the mash tub may not exceed 50 °C in this stage of the process. The mash is maintained under these conditions for a minimum of 30 min while it is being dispersed.

If mash hydrosizing shows that a sufficient degree of disintegration has occurred the dispersion machine is stopped and heating of the mash is started. The mash is heated slowly by about 1 °C per min. Heating is stopped at exactly 64 °C, at which temperature the mash is kept for 5–10 min and then rapidly cooled down to a saccharification temperature of 53–55 °C and the pH is adjusted to 5.3. The mashes are maintained under these conditions for 20–30 min for saccharification. When the saccharification rest is completed, the mash is cooled down to set temperature and fermented as usual.

8.2 Processing Triticale

With an autoamylolytical process it is also necessary to grind the raw material thoroughly or to use a dispersing mash process. An AAQ of at least 95% is required.

The autoamylolytical enzyme system of triticale, in contrast to that of wheat, is rapidly inactivated at 64 °C. However, gelatinization of triticale starch is completed at 60–62 °C, permitting an autoamylolytical processing of triticale.

The required amount of water and/or stillage is pumped into the mash tub and the pH is adjusted to between 5.2 and 5.5. The mash temperature may not exceed 50 °C at this stage of the process. Then the triticale is ground in a hammer mill and also pumped into the mash tub, with BAB added for liquefaction. When milling of the raw material is completed, the mash is maintained at these

conditions for about 30 min. During this time disintegration of triticale is completed by use of the dispersing machine, until mash hydrosizing shows a sufficient degree of disintegration. Afterwards the mash is slowly heated (1 °C per min) to 62 °C as a maximum. The mash is maintained at this temperature for 5–10 min and then rapidly cooled down to the saccharification temperature of 53–55 °C and the pH is adjusted to between 5.3 and 5.5. After a saccharification rest of 20–30 min the mash is corled down to set temperature and fermented as usual.

The gelatinization temperature should be as low as possible to save the autoamylolytical enzyme system of triticale during mashing. Therefore, it is helpful to use microexamination to check starch gelatinization. This examination should be carried out for each new portion of triticale that is worked up. If possible, the gelatinization temperature should be lowered to 60 °C, or, if really necessary, it may be raised to 64 °C for 5 min only.

8.3 Processing Rye

Using the autoamylolytical process for rye, an additional problem is encountered due to the pentosan content of rye (see Sect. 6.3); on the other hand, virtually all varieties of rye have an AAQ higher than 95%.

The pH value of the needed amount of water and/or stillage is adjusted to 5.0 before and after the ground rye is pumped into the mash tub in order to avoid adding pentosanases during the autoamylolytical mashing process. The mash is maintained at 40–45 °C for not more than 20 min at this pH value. If DMP is used, the mash is dispersed under these conditions until a sufficient degree of disintegration is reached. Then it is heated to 60 °C to complete gelatinization of starch for a maximum of 10 min. The gelatinization temperature can be raised to 62 °C if needed for starch gelatinization; however, the mash should not be kept longer than 5 min at this temperature.

Microexamination of the mash should be used to determine whether temperatures higher than 60 °C are needed. To complete saccharification, the mash is cooled to 52–55 °C and the pH is adjusted to 5.3. After a saccharification rest of 20–30 min, the mash is cooled down to set temperature and fermented as usual.

8.4 Saccharification of Raw Materials with Weak Autoamylolytical Activities (Wheat, Corn, Potatoes)

It is not only possible to use the described autoamylolytical activities in triticale to saccharify starch from triticale, it is also possible to use portions of triticale with an AAQ higher than 95% for saccharification of starch from raw

materials such as potatoes, wheat, and corn, with weak or no autoamylolytical activities (Thomas, 1991; Senn et al., 1991). For this purpose it is necessary to prepare two mashes separately in two mash tubs, with one of these mash tubs needing only half the capacity of the other one.

Generally, in one of these mash tubs the gelatinization and liquefaction of the raw material with weak autoamylolytical activities is carried out under the required conditions (Sect. 4.5); the second mash tub is used to prepare the gelatinized and liquefied mash from triticale as described in Sect. 7.2. These mash preparations can be carried out simultaneously, and when both mashes are cooled down to saccharification temperature (53 °C), they are mixed in the bigger mash tub for saccharification.

This process is quite complicated and difficult. But for production of fuel from renewable resources, it is of great importance. Potatoes, e.g., yield twice the amount of starch per ha compared with cereal crops. Also, the cultivation of potatoes and triticale results in a complete rotation of crops and permits agricultural starch production on all the area of arable land. Furthermore, processing in this way avoids the costs for saccharification enzymes. This reduction in costs for ethanol production is important, even if only 20% of the normal concentrations of saccharification enzymes are used (Sect. 8).

Ethanol yields of these mixed mashes depend strongly on the ratio of starch from weak autoamylolytical sources to starch from triticale as shown in Figs. 18 and 19. As shown for potatoes and corn, it is necessary to have 50% of the starch from triticale in these mixed mashes to reach optimum ethanol yields of more than 64 lA per 100 kg FS.

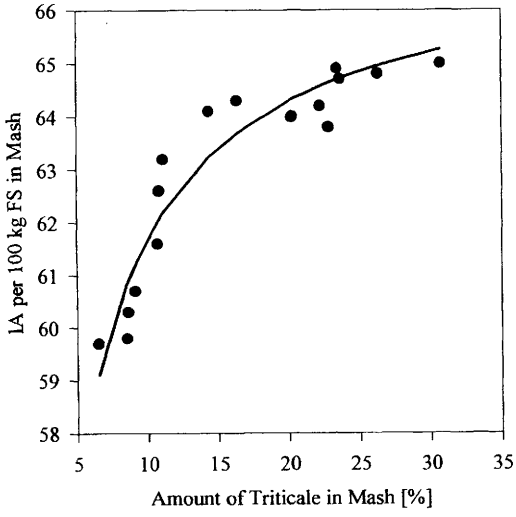

Fig. 18. The relationship between ethanol yields from potatoes using triticale for saccharification and the amounts of triticale in the mash.

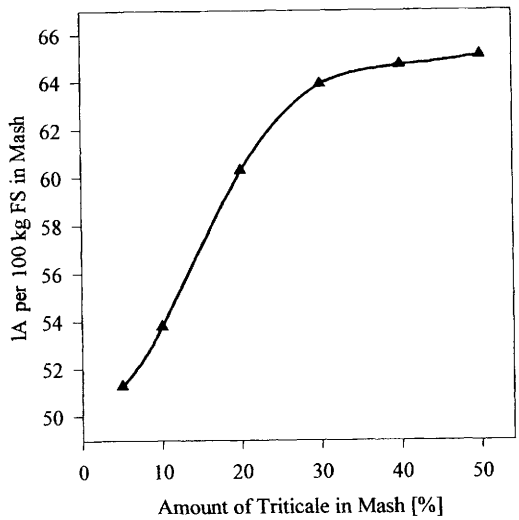

Fig. 19. The relationship between ethanol yields from corn, using triticale for saccharification, and the amounts of triticale in the mash.

9 Yeast Mash Treatment

The yeast needed for fermentation is normally cultivated in the distilleries as a separately fermented yeast mash, usually using the sulfuric acid method. The volume of yeast mash needed daily in distilleries ranges from 5–10% of the mash volume. To accelerate the fermentation and to suppress contamination in fermentations, 10% of yeast mash should be used.

A dried distillers' pure-culture yeast is usually used. This distillers pure-culture yeast is added to sweet mash which was pumped into the yeast mash tub. Then the pH is adjusted to between 3.0 and 3.5 and the yeast mash is cooled to 20–22 °C. During cultivation for about 18–24 h the temperature of the mash should not exceed 27 °C. This can be regulated by changing the set temperature of the yeast mash or by using the cooling facilities of the yeast mash tub.

Generally, the extract content of yeast mashes during yeast mash fermentation should decrease to about 50% of the extract content of the sweet mash. Thus the fermented (ripened) yeast mash should have an extract content of 7–10% mas when it is added to sweet mashes. This guarantees a rapid start of the fermentation and ensures an optimum protection against contaminations.

The use of stillage recycling in ethanol production also leads to an accelerated fermentation in yeast mashes, and, therefore, it may be necessary to cool yeast mashes more intensively. However, stillage recycling allows the lowering of pH values in yeast mashes down to 2.5. This pH does not effect on yeast growth, but leads to an effective protection against bacterial contamination in

yeast mashes. But the pH value is not lowered below 3.0 at the start. To reach a pH of 2.5, the pH-value of yeast mash should be successively lowered during the first three days of yeast cultivation.

The pH adjustment of yeast mashes is done by adding concentrated sulfuric acid to the mash. Therefore, this method is called the sulfuric acid method.

10 Fermentation

10.1 Batch Fermentation

The fermentation of mashes is normally carried out in cylindrical fermentation vessels. These fermenters should be

- made from stainless steel,
- standing cylindrical fermenters, designed higher than the diameter of the vessel,
- fitted with manholes at the bottom of the cylindrical part of fermenter and at the top,
- fitted with an inspection glass above the bottom manhole, a thermometer and a carbon dioxide collection tube at the top.

If the volume of the fermenter is greater than 40 m^3 it is useful to fit it with a cooling coil, which must be designed to enable a rapid and effective cleaning. Such a cooling facility inside the fermenters is quite effective and permits a better temperature control during the fermentation. While the fermentation temperature in smaller fermenters or in fermenters without any cooling facility is regulated by the selection of set temperature, fermentation can be started at a higher set temperature if a cooling coil is installed in the fermenter. Using, e.g., a fermenter with a volume of about 30 m^3, the set temperature has to be reduced to 20–22 °C. If a cooling coil is installed into the fermenter, set temperature of 28–30 °C can be maintained. These higher set temperatures lead to an accelerated start of the fermentation and, as a consequence, the duration of the fermentation is reduced as well as the risk of contamination. If, however, the yeast mash used in the fermentation is not absolutely free from contamination, higher set temperatures result in an increased risk of contamination. A cooling coil in fermenters reduces the consumption of cooling water during the mashing process, while water consumption during the fermentation increases.

A perforated water tubing is often installed on the top so that water can be sprinkled on the surface of the fermenters. But this kind of spray cooling is quite ineffective and leads to encrustations on the surface of fermenters.

Fermentation can be started with yeast mash in two ways. The first possibility is the addition of yeast mash to sweet mash in the mash tub at about 33 °C.

This inoculated mash is then cooled down to set temperature and pumped to the fermenter and the yeast mash tub again. The second way to start fermentation, usually used if the distillery is equipped with a heat exchanger, is to pump about 90–95% of fermented (ripened) yeast mash into the fermenter. After this, or while the yeast mash is being pumped into the fermenter, the sweet mash from the mash tub is pumped into the fermenter by passage through the heat exchanger to reach set temperature. And the yeast mash tub is refilled too, with sweet mash cooled down to set temperature.

Generally the set temperature is selected to reach a fermentation temperature of 34 °C after about 24 h; the fermentation temperature should not exceed 36 °C during the entire fermentation. A cooling facility may be used in fermenters to maintain the fermentation temperature at 34 °C after this temperature is reached.

In practice it is important to follow the course of the fermentation analytically. Thus extract content, pH and temperature should be measured and documented at least once a day. Microscopic examinations should also be carried out daily.

Normally, after 24 h of fermentation about 50% of fermentable extract contained in the mashes is metabolized and the ethanol content has reached about 4% vol. At the start of fermentation, the pH is 5.2, which decreases over about 24 h to 4.6–4.8. During further fermentation the pH decreases to about 4.2–4.5. At the end of fermentation the pH increases by about 0.2 pH units if the mash is free from contamination. If the pH falls below 4.0, this is normally due to contamination.

It is impossible to give generally valid data concerning extract contents (degree of fermentation) as a criterion for the completion of the fermentation. These data depend on the mashing process used (pressure cooking or pressureless), the raw material used, the ratio of stillage recycling used in the process, and the extract content of sweet mashes. Therefore, it is essential to follow fermentations using analytical methods in each distillery. Reliable data for a certain distillery can be obtained, if these determinations provide consistent data, and if the same mashes yield more than 64 lA per 100 kg FS. In practice, the degree of fermentation varies between −1.5 and +4 %mas. Tab. 14 shows the data for the classical HPCP (Kreipe, 1981).

Tab. 14. Degree of Fermentation (HPCP) (Kreipe, 1981)

Raw Material	%mas
Potatoes	0.7–1.4
Corn, sorghum	< 0.0
Rye	0.5–1.0
Wheat	0.0–0.5
Manioc	< 0.0

Stillage recycling, used in the mashing process, leads to an additional important effect in the fermentation (Senn, 1988). The start of the fermentation is clearly accelerated if stillage recycling is used; corn mash prepared without stillage has a long lag phase of more than 4 h. If stillage amounts to 50% of the process liquid, the lag phase is reduced to 1.5–2 h. Using exclusively stillage as process liquid, the fermentation starts with a small or no lag phase. This rapid start of fermentation using stillage recycling clearly reduces the risk of contamination. Not only is the start of the fermentation accelerated, but also the duration of the fermentation is reduced. Thus, processing wheat, rye, or triticale, it is possible to conduct a fermentation of only 40 h without problems. With a set temperature of, e.g., 28 °C, it is possible to finish this fermentation within 30 h. Fermentation of corn mashes takes about 60 h, while fermentation of corn grain silage takes 40 h.

10.2 Suppression of Contaminants

In recent years more and more distilleries have been affected by the formation of propenal (acrolein) in mashes during fermentation. Propenal is enriched in raw distillates and results in a drastic reduction in product quality. Therefore, it was necessary to develop methods to avoid formation of propenal in mashes. Krell and Pieper (1995) tried to avoid propenal (acrolein) formation right from the beginning of the fermentation while Butzke and Misselhorn (1992) tried to reduce the propenal content in raw distillates during the distillation process by chemical oxidation.

Since the formation of propenal (acrolein) in mashes is due to the growth and metabolism of sporulating bacteria (Krell and Pieper, 1995), it is necessary to create conditions in mashes, that lead to pasteurization of the mashes during the mashing process and that prevent germination of bacterial spores during the fermentation. Therefore, mashes are kept at more than 65 °C for a minimum of 30 min to reduce the microbial count as far as possible. After liquefaction and pasteurization, the mashes are cooled to saccharification temperature. But, before 60 °C are reached, the pH of the mashes is adjusted to 4.0 to avoid germination of spores during further processing, using concentrated sulfuric acid. This kind of specific acidification of mashes was found to stop propenal (acrolein) formation effectively in a German distillery processing 15 t of potatoes per fermenter, and at the same time, ethanol yields increased from 61.9 lA per 100 kg FS (n = 89) to 64.2 lA per 100 kg FS (n = 104).

Specific acidification of mashes is very effective in avoiding contamination of mashes due to the germination of spores. But there is only a weak effect in avoiding contamination that results from contaminated mash residues in pipes, heat exchangers and other regions that are difficult to clean. But, if the mashes have a pH value of 4.0 it is possible to use sulfur dioxide (SO_2) to reduce the

risk of contamination drastically, as done in wine making technology (Krell and Pieper, 1995). The use of SO_2 permits the processing of all starchy raw materials virtually free from contamination, if the following steps are carried out:

- acidification of the mash to a pH of 4.0 above 60 °C using concentrated sulfuric acid (about 1 L m^{-3} mash is needed),
- acidification of yeast mash to a pH of 2.5.

If these measures are not sufficient to suppress contamination in mashes, an additional step is used:

- addition of SO_2 (50 g m^{-3}) or $K_2S_2O_5$ (100 g m^{-3}) to the mashes.

11 Distillation

11.1 Distillation of Raw Spirit from Mashes

Under the conditions of the German state monopoly on ethanol, it is necessary to distill a raw spirit from fermented mashes with an ethanol concentration of between 82% and 87% by volume, if it is delivered to the state monopoly. The stills used in distilleries to produce raw spirit for the state monopoly nowadays are completely manufactured from stainless steel. To reach the required ethanol concentration in raw spirit, a distillation apparatus is needed, that consists of four sections:

- the mash column, to distill off ethanol from the mash,
- the enriching section, to increase ethanol concentration in the raw distillate,
- the dephlegmator, to enrich ethanol in the vapor phase by partial condensation,
- the cooling section, to condense and cool down the ethanol vapor.

The mash column (Fig. 20) can either be heated by direct steam injection or indirectly, using a heater assembly which consists of heating pipes or heating plates. Mash columns are usually constructed with bubble plates because of the solids contained in mashes. Depending on the efficiency of distillation, bubble plates should be at least 300 mm apart. It is sufficient to use a single bubble cap on the distillation plates if the diameter of the column is not larger than 800 mm. When using columns with a diameter of more than 1 m, it is necessary to fit the plates with more bubble caps to maintain distillation efficiency. The diameters of these plates should not be too small, to avoid clogging if several bubble caps are used on a distillation plate. At the front stills should be fitted with sight glasses, and, if the column diameters exceed 700 mm, stills should additionally be fitted on the backside with openings for cleaning between each

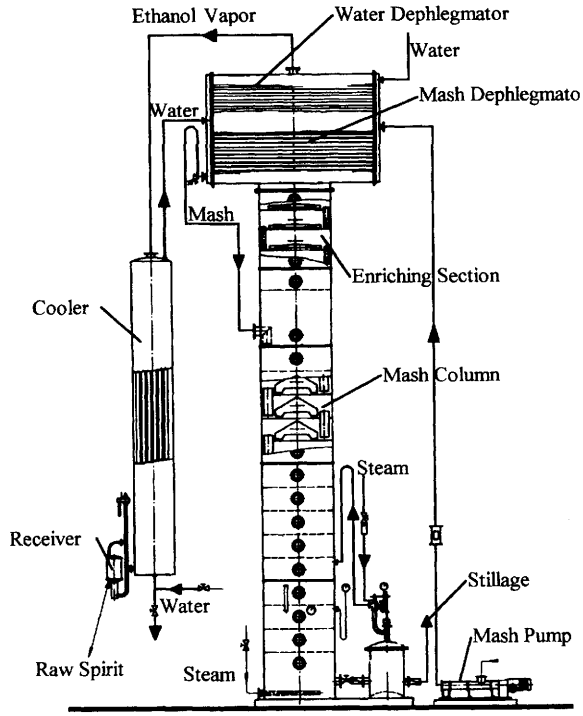

Fig. 20. Mash distillation column; mash and water dephlegmator (Kreipe, 1981).

plate (Kreipe, 1981). To ensure that the stillage leaving the mash column is free from ethanol, about 13–15 bubble plates are needed.

The enriching section of raw spirit stills is normally fitted with 4–6 bubble plates, with a distance of about 200 m between them. It is important to have a sufficient distance between the very top bubble plate of the mash column and the bottom plate of the enriching section to avoid that foam or mash is carried over to the enriching section by the vapor stream. The enriching section should also be fitted with sight glasses and cleaning openings, as described above.

Lying rectangular dephlegmators are in use to obtain an effective enrichment of ethanol in the vapor phase. The inside of these dephlegmators consist of a tube system, which is easy to clean. The mash that is distilled passes two thirds of these pipes. One third is used as a dephlegmator system using cooling water. In this manner, passing through the tubing system of the dephlegmator, the mash is heated to about 80 °C, while it is also used as a cooling agent for dephlegmation. The topmost third of the tubing system in these types of dephlegmators is used as a water dephlegmation system, to complete the dephlegmation at an enrichment ratio needed to reach a distillate with a sufficient ethanol concentration. A sufficient ethanol concentration of raw spirit is reached,

if the ethanol vapor phase leaving the dephlegmator has a temperature of 78 °C or lower.

In 1992, a new generation of dephlegmators was developed, designed as pure mash dephlegmators (Fig. 21). These dephlegmators are cylindrical with a diameter that is somewhat greater than the diameter of the mash column and the enriching section. Internally there is a doubled heating coil through which the mash passes, and a water container situated in the middle of the heating coil. The length of the coil is sufficient to lead to the necessary enrichment ratio, if only fermented mash is used as a cooling agent in dephlegmation. The water container in this new kind of dephlegmator is used only as a reserve facility for extreme situations such as unusually low ethanol concentrations in mashes.

After leaving the top of the dephlegmator, the ethanol vapor is condensed, and the raw spirit obtained is cooled to about 20 °C, usually by using tubular coolers. Often the cooling water needed for distillation is first used as a cooling agent in the tubular cooler and then, after it has warmed up there, it is used in the dephlegmator. This saves some cooling water, but regulation of the still is easier and more effective if cooling water used in the cooler and the dephlegmator is separately controlled. It is pointless to couple cooling and dephelegmation, since the cooling of the product is without any influence on the deph-

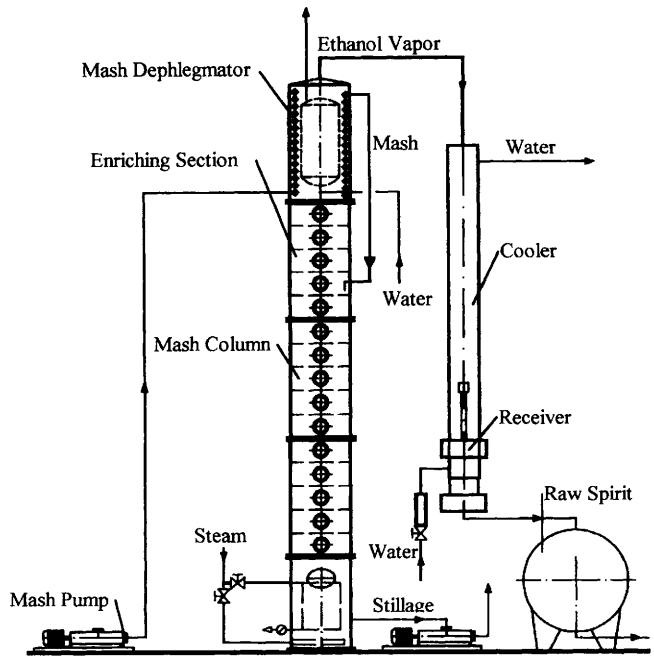

Fig. 21. Mash distillation column; pure mash dephlegmator (J. Carl, D-73002 Göppingen).

legmation process. In modern stills, both processes consuming cooling water are separately controlled. The energy consumption of the mash still described above amounts to about 2 kg of vapor per lA (Kreipe, 1981).

11.2 Rectification of Product Spirit from Raw Spirit

Rectification of raw spirit, when distilleries produce their own potable spirit, is normally carried out using a batch rectifying apparatus. These rectifying stills are manufactured completely from copper. Only the pipes collecting the top fraction of the heads are from stainless steel to avoid corrosion of copper. These rectification stills (Fig. 22) in principle consist of:

- a reboiler (still pot),
- a rectifying column,
- a main condenser,
- an aldehyde condenser,

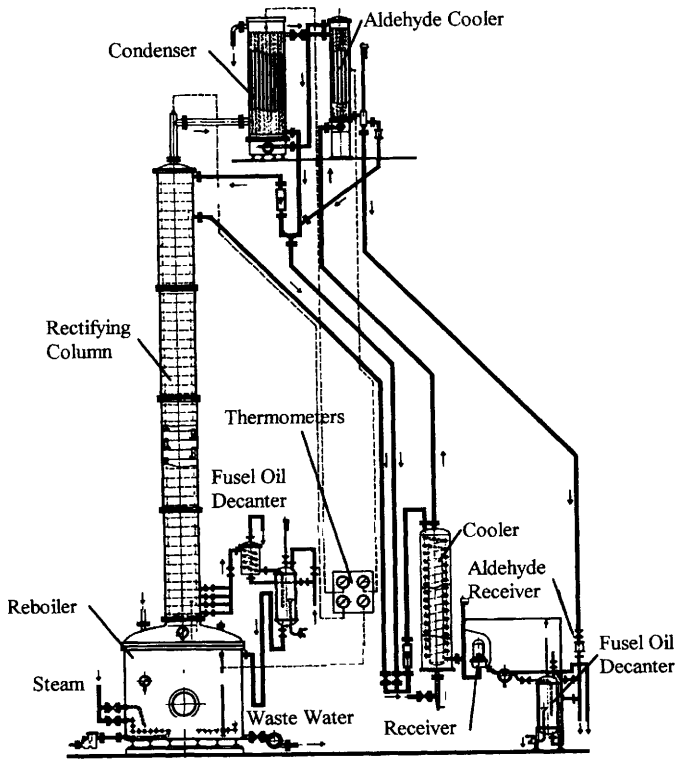

Fig. 22. Batch rectifying apparatus (Kreipe, 1981).

- a product cooler,
- a receiver for the top of the heads,
- a receiver for the heads, the product fraction and the tailings,
- a fusel oil decanter.

Before raw spirit is rectified, it is pumped into the reboiler and diluted there to about 40% ethanol by volume. The reboiler should be only filled up to about 80% of its volume. To heat up diluted raw spirit, the reboiler is fitted with a heating coil for indirect heating. In addition, the reboiler is fitted with pressure and temperature indicators, a sight glass, a manhole and sometimes a still dome.

The rectifying column consists of at least 45 sieve plates in order to enrich ethanol to at least 94% by volume or more. The ethanol vapor leaves the rectifying column at the top and is led to the main condenser. The main condenser and the aldehyde condenser are manufactured mostly as tubular coolers. The less volatile components condense in the main condenser and flow back to the top of the rectifying column. The highly volatile components are led to the aldehyde condenser and condense there to flow back to the top of the column. Alternatively both condensation products can be drawn off from the condensers and collected in separate receivers. At the beginning of rectification, the top of the heads are drawn off from the aldehyde condenser and are collected separately using the top of the heads receiver. After separation of these tops, the product is drawn off as liquid from the rectifying column. A plate, some plates below the top plate, is used for this purpose. During further rectification, this product is fractionated into heads, product, and tailings. The tailings are fed to the fusel oil decanter where they are diluted with cold water to separate the fusel oils.

Vapor and water pressure must be precisely controlled to obtain a sufficient degree of rectification. Furthermore, it is necessary to install flow meters for steam used in heating and for cooling water. Flow meters are also needed in product and reflux tubes (downpipes) to adjust the reflux ratio during the rectification process, since rectification efficiency depends greatly on the reflux ratio.

In practice, the rectifying stills in use are of various designs. It is possible to construct a rectifying column directly above or beside the reboiler. Or, e.g., it is feasible to install a second product cooler, to permit cooling of the product fraction separate from the cooling of heads and tailings. Another possibility is the installation of draining tubes at the bottom of the rectifying column to draw off fusel oil-containing fractions into a second fusel oil decanter. Further information on the design of rectifying plants and rectification theory have been reported by Kreipe (1981) and Kirschbaum (1969).

This kind of batch fractionated rectification leads to an energy consumption of about 2.5 kg of vapor per lA. Then energy consumption would increase to 5–6 kg vapor per lA, if distillation and rectification are carried out separately.

11.3 Distillation and Rectification of the Alcohol Product from Mashes

Because of the energy consumption in distillation and rectification, it is necessary to use a continuous and combined distillation and rectification still in large-scale plants for ethanol production. Kreipe (1981) and Kirschbaum (1969) have reported details for such plants. Investment in such plants is too expensive, and their capacities are too high for distilleries working in the classical tradition. In 1993, a distillation and rectification still was developed that may be of interest to distilleries producing their own potable neutral product distillates. This still can also be used at a large scale.

As shown in Fig. 23, fermented mash is pumped through the cooling coil of the dephlegmator on top of the rectification column and is heated up there. The heated mash is fed to the degassing section of the mash column, and while flowing downwards, ethanol is stripped from the mash. The stillage leaves the mash colunm through the bottom. The degassing section is used to strip high-volatile components which are led to a dephlegmator. The phlegma is fed back to the mash column and the volatile components are condensed and cooled in a tubular cooler, from where the headings are drawn off into a separate receiver.

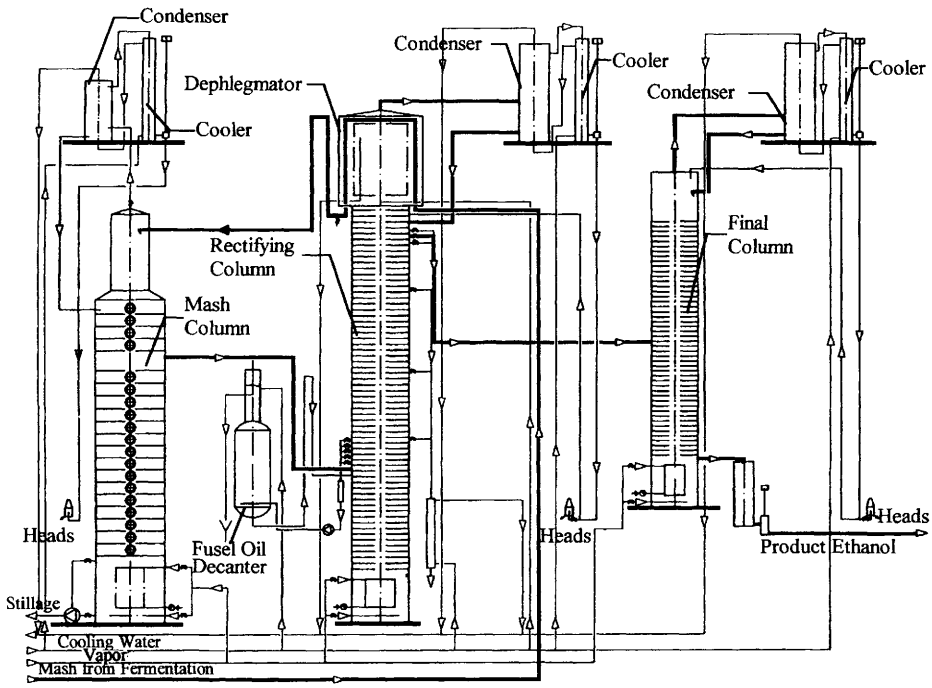

Fig. 23. Continuous distillation of product ethanol from mash (J. Carl, D-73002 Göppingen).

The ethanol vapor is fed to the rectification column, where rectification takes place as described above, but continuously. From a plate near the top of the rectification column, product spirit is fed to an end column as a liquid draw. This end column is used to separate residual aroma from the product spirit as far as possible to yield virtually neutral spirit with about 96% by volume.

Energy consumption for this type of continuous distillation and rectification also amounts to 5 kg of vapor per lA, but the capacity is much higher compared to using a batch rectifying still.

12 Stillage

12.1 Stillage as a Feedstuff

About 90–140 L of stillage are obtained from each hectoliter of fermented mash depending on the distillation equipment. For a continuous mash distillation stillage amounts to 1,100–1,400 L per 100 lA.

While in the USA more than 85% of grain stillage is dried, in Europe stillage is fed as a liquid as it is obtained from distillation. This is due to the smaller scale of the distilleries which does not warrant the costs of investment for stillage drying plants. In addition to the costs, drying of stillage consumes the same amount of energy as is needed for ethanol production itself (Pieper, 1983). It should also be considered that it is easier to dry corn stillage than it is to dry stillages from rye, potato, or wheat mashes.

Before stillage is dried, it has to be separated. Concentration of stillage is more successful, the lower the solids content. So before drying, stillage is first separated into thin stillage and solids, called grains, using sieves or decanters. When sieves are used, the grains obtained are about 15–20% DS and can be further dried to about 30–35% DS with a certain press. The grains obtained from a decanter reach a DS of about 25–30%. Thin stillage is further clarified using centrifuges or settling basins. The centrifuge cake obtained from centrifuges has about 20% of DS, of which 50% consists of protein. The clarified thin stillage is then concentrated up to 35–40% DS, and in some cases to 60% DS. Using spray dryers or drum dryers this concentrated sirup is dried to about 90–95% DS. If only clarified thin stillage is dried, the product is called "Dried Distillers' Solubles" (Pieper, 1983).

To yield a product containing the total DS from the original stillage, the concentrated sirup of thin stillage has to be homogeneously mixed with the solid fraction. This is a difficult process, that is only successful, if previously dried and hot stillage is also added to this mixture. "Distillers' Dried Grains with Solubles" are obtained when this mixture is dried in special drum dryers to 92–94% DS.

Stillage and stillage products used as feedstuff can be classified as follows (Pieper, 1983; Ogowi et al., 1996):

- Whole stillage: stillage obtained from distillation.
- Thin stillage: liquid phase obtained by sieving, centrifugation, or settling.
- Grains: solid phase obtained by sieving, centrifugation, or settling.
- Centrifuge cake; obtained by clarification of thin stillage.
- Semi-solid distillers' solubles: concentrated thin stillage produced by evaporation.
- Dried distillers' solubles: dried concentrated thin stillage.
- Distillers' dried grains, light grains: dried grains, obtained by sieving, centrifugation or settling.
- Distillers' dried grains with solubles, dark grains: mixture of semi-solid distillers' solubles and grains, dried with a drum dryer or mixture of light grains and dried distillers' solubles.

As stillage is a very important feedstuff, the nutrient content of stillages of different origins, which varies widely, is shown in Tabs. 15–21. Data for digestibility coefficients are given for information only.

Some additional data of the composition of stillage from wheat and corn, obtained using DMP with stillage recycling, are shown in Tabs. 22 and 23. This process yields two fractions of stillage, which are stored in a sedimentation tank and settled there. The thin stillage fraction is clarified and used in the process again, and amounts to about 50% of total stillage. The other fraction is thick stillage, which contains virtually all the solids. It is used as feedstuff or fertilizer.

Tab. 15. Nutrient Content [% of DS] and Digestibility [%] of Stillage from Potatoes (Kling and Wöhlbier, 1983)

Component	Whole Stillage n=27		DDGS n=7	DDS n=1
OS	87.4±3.1		85.6±1.7	91.8
Raw protein	27.0±3.4		27.3±2.1	11.7
Raw fat	2.7±3.4		2.1±1.3	2.1
Crude fiber	8.1±3.8		9.8±3.4	9.6
NNE	49.9±6.8		46.5±3.6	68.5
ASh	12.6±3.1		14.4±1.7	8.2
DS	ca. 6%		ca. 90%	ca. 93%
Digestibility	Cow n=2	Pig n=2	Cow n=5	Pig n=7
OS	63	70	72	68
Raw protein	56	58	58	47
Raw fat	50	72	40	56
Crude fiber	37	77	74	66
NNE	71	79	80	84

Tab. 16. Nutrient Content [% of DS] and Digestibility [%] of Stillage from Wheat (Kling and Wöhlbier, 1983)

Component	Whole Stillage n = 1	DDGS n = 2	DDS n = 6	Light Grains n = 6
Os	91.4	92.5	91.8 ± 2.5	97.6 ± 0.2
Raw protein	34.8	32.0	41.2 ± 4.6	36.4 ± 7.5
Raw fat	2.2	6.4	0.9 ± 0.3	4.4 ± 0.8
Crude fiber	3.4	6.5	2.4 ± 0.3	13.4 ± 1.7
NNE	51.0	44.6	47.2 ± 3.1	43.9 ± 4.7
Ash	8.6	7.5	8.2 ± 2.5	2.4 ± 0.2
DS	ca. 4.2%	ca. 91%	ca. 89%	ca. 90%
Digestibility	Cow n = 1	Cow n = 1	Pig n = 4	
OS	58	57	67	
Raw protein	54	55	68	
Raw fat	50	47	100	
Crude fiber	43	44	20	
NNE	62	61	72	

Tab. 17. Nutrient Content [% of DS] and Digestibility [%] of Stillage from Rye (Kling and Wöhlbier, 1983)

Component	Whole Stillage n = 1	DDGS n = 3	DDS n = 3	Light Grains n = 4
OS	96.5	97.0	91.5	92.4 ± 10.8
Raw protein	42.4	22.0	38.6	25.9 ± 2.2
Raw fat	3.5	5.1	0.8	6.5 ± 0.4
Crude fiber	5.9	15.1	2.5	13.9 ± 2.0
NNE	44.7	54.8	49.6	46.1 ± 9.8
Ash	3.5	3.0	8.5	7.7 ± 10.8
DS	ca. 8%	ca. 89%	ca. 95%	ca. 91%
Digestibility	Sheep n = 2	Sheep n = 4		
OS	65	51 ± 8		
Raw protein	60	59 ± 7		
Raw fat	58	62 ± 3		
Crude fiber	50	50 ± 18		
NNE	71	49 ± 6		

12.2 Stillage as a Fertilizer

If stillage is used as a fertilizer it should be spread as fresh stillage. Placing of fresh stillage is virtually odorless. If stillage cannot be spread fresh, and it is necessary to store it, preserving agents have to be added. Stillage can create a very unpleasant smell if it is stored without addition of preserving agents.

Tab. 18. Nutrient Content [% of DS] and Digestibility [%] of Stillage from Barley (Kling and Wöhlbier, 1983)

Component	Whole Stillage n=8	DDGS n=2	DDS n=8	Light Grains n=1
OS	97.9±0.2	94.5	94.5±0.6	96.9
Raw protein	31.3±0.6	22.7	26.8±4.6	19.8
Raw fat	10.2±1.2	4.0	5.2±2.2	8.4
Crude fiber	13.7±0.8	11.0	10.4±5.0	18.6
NNE	42.7±1.4	56.8	52.1±8.7	50.0
Ash	2.1±0.2	5.5	5.5±0.6	3.1
DS	ca. 26%	ca. 93%	ca. 91%	ca. 93%
Digestibility	Sheep n=4			
OS	66±2			
Raw protein	81±1			
Raw fat	88±1			
Crude fiber	34±8			
NNE	63±2			

Tab. 19. Nutrient Content [% of DS] and Digestibility [%] of Stillage from Corn (Kling and Wöhlbier, 1983)

Component	Whole Stillage n=12	DDGS n=24	DDS n=24	Light Grains n=14
OS	95.3± 1.3	95.2±2.2	89.8±7.7	96.7±1.5
Raw protein	25.5± 7.0	28.5±5.3	28.2±5.5	26.6±5.8
Raw fat	11.7± 3.6	7.7±3.4	5.4±3.0	7.9±7.8
Crude fiber	10.6± 4.7	11.9±3.2	5.3±3.4	12.3±2.9
NNE	47.6±12.5	47.2±6.5	51.2±5.5	49.9±8.8
Ash	4.7± 1.3	4.8±2.2	10.1±7.7	3.3±1.5
DS	ca. 8.5%	ca. 90%	ca. 92%	ca. 92%

Digestibility	Sheep n=2	Cow n=1	Pig n=4	Cow n=2	Chicken n=1	Chicken n=1
OS	67	72	80	75	69	58
Raw protein	64	67	78	73	80	78
Raw fat	89	91	92	81	90	56
Crude fiber	59	69	81	32	0.0	36
NNE	71	75	91	89	60	51

With respect to use of stillage from potatoes, Matthes (1995) calculated the following nutrients content of fertilizer:

- 2.7 kg m^{-3} N
- 1.1 kg m^{-3} P_2O_5
- 4.2 kg m^{-3} K_2O

Tab. 20. Nutrient Content [% of DS] of Stillage from Sorghum Grain (Kling and Wöhlbier, 1983)

Component	Whole Stillage n=8	DDGS n=2	DDS n=2
OS	94.7±1.3	93.7	86.2
Raw protein	29.8±3.4	26.6	21.2
Raw fat	8.6±2.7	7.3	6.6
Crude fiber	8.8±1.7	9.1	3.8
NNE	47.5±6.4	49.9	54.7
Ash	5.3±1.3	6.3	13.8
DS	ca. 17%	ca. 93%	ca. 92%

Tab. 21. Nutrient Content [% of DS] of Stillage from Manioc (Kling and Wöhlbier, 1983)

Component	Whole Stillage n=1	DDGS n=1	DDS n=1
OS	93.2	90.7	91.1
Raw protein	14.4	12.3	28.4
Raw fat	1.8	1.6	5.3
Crude fiber	2.8	5.6	2.1
NNE	74.2	71.2	55.3
Ash	6.7	9.3	8.9
DS	ca. 3.3%	ca. 92%	ca. 95%

Tab. 22. Composition of Stillage [% of OS] from Wheat Obtained from DMP with Stillage Recycling

Component	Whole Stillage n=2	Thin Stillage n=2	Thick Stillage n=2
Raw protein	2.40	1.34	3.38
Raw fat	0.35	0.00	0.82
Crude fiber	0.43	0.004	1.14
NNE	2.26	1.68	4.15
Ash	0.40	0.38	0.41
DS	6.2	3.4	9.9

Tab. 23. Composition of Stillage [% of DS] from Corn Obtained from DMP with Stillage Recycling

Component	Whole Stillage n=2	Thin Stillage n=2	Thick Stillage n=2
Raw protein	31.5	21.2	41.4
Raw fat	15.1	–	14.3
Crude fiber	12.6	–	9.0
NNE	34.3	–	30.3
Ash	6.5	–	5.0
DS	3.7	2.1	5.8

Further the use of fresh potato stillage results in a cost saving of 0.93 DM per hlA produced in a distillery, compared with the use of mineral fertilizers. But if stillage has to be stored there are additional costs that amount up to 5.45 DM per hlA (Matthes, 1995).

For the use of fresh grain stillage from a distillery processing with stillage recycling (settling of stillage in a sedimentation tank), the savings are greater because the amount of stillage is halved, and the protein content of thick stillage is about 50% higher compared with whole stillage obtained from the distillation. This reduces fertilizer costs compared with the fertilizer from stillage of potatoes. In addition, if stillage has to be stored, one has to store half the amount only, thereby reducing the storage costs.

There are some advantages to the use of stillage as fertilizer compared with mineral fertilizers. Stillage contains only a part of the total nitrogenous nutrients in the form of immediately available nitrogen. Most of the nitrogen is fixed in proteins, and is slowly mineralized during the growth period. Thus, there is only a low risk of rinsing out nitrogen to the ground water. But further tests of the use of stillage as a fertilizer are still necessary.

13 Analytical Methods

13.1 Introduction

It is absolutely necessary to follow conversion and distillation processes using analytical methods. There is no other way to ensure an effective and careful process and to avoid contamination and ethanol losses due to incomplete conversion. The most important analytical methods used in distilleries are described in this section.

13.2 Analysis of Raw Materials

13.2.1 Starch Content of Potatoes

In practice it is very difficult to determine the exact starch content of potatoes, since it is virtually impossible to prepare an average sample under practical conditions. Furthermore, the starch content varies among individual potatoes.

Special balances are used to determine the density of potatoes. In this procedure exactly 5,050 g of wet potatoes are weighed out, and then these potatoes are weighed again under water. The density of potatoes can be calculated using Eq. (2):

$$\begin{aligned} \text{Density} &= \frac{\text{Absolute weight}}{\text{Volume}} \\ &= \frac{\text{Absolute weight}}{\text{Loss of weight}} \\ &= \frac{5{,}050\ \text{g}}{5{,}050\ \text{g} - \text{Loss of weight}} \end{aligned} \tag{2}$$

The relation between the density of potatoes and their dry substance or starch content can be regarded as constant. Therefore, it is possible to calculate the starch content of potatoes using the tables first calculated by Behrend (Kreipe, 1981; Adam et al., 1995). The most widely used balances for this purpose are those of Parow or Eckert. Both balances are scaled to weigh out 5,050 g potatoes, and a second scale directly shows the starch content when the potatoes are weighed under water.

This method is not very exact, but good enough for ethanol production purposes; these balances are also often used in the starch and food industry. If large potatoes are weighed with these balances, it is necessary to cut through them, since large potatoes often have internal cavities.

13.2.2 Starch Content of Grain

Normally the starch determination is carried out using polarimetric methods. These methods consist of the following steps:

- release of starch from cell material and solubilization of starch;
- separation of optically active substances, that may interfere with the measurement of starch;
- measurement of the angle of polarization due to the concentration of solubilized starch.

These methods have been described by Ewers (1909), Earl and Millner (1944), and Clendenning (1945). It was shown by Senn and Pieper (1987) that a variation in clarifying agents used for the separation of optically active substances interferes with the measurement of starch. Therefore, it was suggested by Senn and Pieper (1987) to determine instead the content of fermentable substances in grain for ethanol production purposes.

13.2.2.1 Determination of Fermentable Substance in Grain (FS)

The fermentable substance (FS) is defined as the sum of the glucose and maltose contents of the raw material, calculated as starch, that can be determined

using HPLC after the raw material is completely digested and dispersed as well as liquified and saccharified by addition of technical enzymes.

The determination of FS is carried out using the following procedure:

- 10 g of thoroughly milled grain are weighed in a mash beaker of a laboratory masher with a precision of 1 mg.
- 300 mL of H_2O are added.
- The pH is adjusted to 6.0–6.5.
- Each charge is dispersed for 1.5 min, using a laboratory dispersing machine (e.g., ULTRA TURRAXT™).
- Then the mash beaker must be placed in the laboratory masher and is stirred there.
- 0.2 mL of a thermostable α-amylase from *B. licheniformis* is added (e.g., Termamyl 60 L, NOVO-Industri, Kopenhagen).
- The laboratory masher is heated up to 95 °C.
- While stirring the charges are maintained at 95 °C for 60 min.
- During this rest, after about 30 min, the charges are dispersed again for about 3 min.
- After this rest the masher is cooled to 52–53 °C (temperature inside the beakers).
- The pH is adjusted to 5.0.
- For saccharification the following enzymes are added:
  - 0.2 mL fungal α-amylase from *A. oryzae* (e.g., Fungamyl L 800L, NOVO-Industri, Kopenhagen),
  - 2.0 mL glucoamylase from *A. niger* (e.g., Optisprit-L, Solvay Enzymes, Hannover),
  - 0.1 g glucoamylase from *Rhizopus* sp. (e.g., Optilase G 150, Solvay Enzymes, Hannover).
- A saccharification rest is done over night to complete saccharification, for which the beakers have to be covered.
- Then the charges are quantitatively rinsed into a 1,000 mL measuring flask.
- The measuring flask is kept at a constant temperature and exactly filled up.
- The sample is then filtered using folded filters (MN 615 $^1/_4$).
- The sample is filtered again using a 0.45 μm membrane filter.
- 20 μL of this sample are then injected into the HPLC system.

For the determination of glucose and maltose the following chromatographic equipment and conditions are used:

- Pump: Bischoff, Model 2200
- Chromatographic column: Biorad, HPX 87 H, 30 cm
- Temperature: 50 °C
- Eluent: purified water
- Flow rate: 0.7 mL min^{-1}

- Detection: RI-Detector, ERC-7510
- Sample amount: 10 μL

Using the results obtained from the HPLC determination, the FS content of the raw materials is calculated following Eq. (3):

$$FS = \frac{\text{Glucose } [g\ L^{-1}] \cdot 0.899 + \text{Maltose } [g\ L^{-1}] \cdot 0.947}{\text{Weighed portion of raw material } [g] \cdot 100} \quad (3)$$

This method for the determination of FS can be used for all starch containing raw materials, weighing about 1–7 g of starch into the mash beakers. This method can also be used to examine stillage. Organic acids and alcohols can also be determined with a Biorad HPX-87-H column. Therefore, the analysis of stillage can be used to examine the quality of the ethanol production processes, showing, e.g., if there is residual FS in the stillage, contamination of mashes (organic acids), and ethanol loss in distillation.

13.2.3 Autoamylolytical Quotient (AAQ)

For processing wheat, rye, or triticale, it is important to know the AAQ, which gives information on the activity of the autoamylolytical enzyme system. The AAQ is defined as the percentage yield of ethanol obtained without the addition of saccharifying enzymes, compared with the ethanol yield with addition of an optimum combination of technical enzymes (see Eq. (1)).

In order to calculate the AAQ, it is necessary to carry out fermentation experiments in the laboratory, using the following procedures to examine wheat, rye, and triticale.

Fermentation test using technical enzymes:

- 80.0 g of thoroughly milled grain are weighed in a mash beaker of a laboratory masher.
- 300 mL of H_2O are added.
- The pH is adjusted to 6.0–6.5.
- Each charge is dispersed for 1.5 min, using a laboratory dispersing machine (e.g., ULTRA TURRAX™).
- Then the mash beaker is placed in a laboratory masher and stirred.
- 0.65 mL of a thermostable α-amylase from *B. licheniformis* is added (e.g., Termamyl 60 L, NOVO-Industri, Kopenhagen).
- The laboratory masher is heated to the liquefaction temperature of 65 °C.
- While stirring, the charges are maintained at liquefaction temperature for 30 min.

- After this rest the masher is cooled to 52–53 °C (temperature measured inside the beakers).
- The pH is adjusted to 5.0–5.2.
- For saccharification the following enzymes are added:
  - fungal α-amylase from *A. oryzae* (0.1 mL kg^{-1} FS, e.g., Fungamyl L 800L, NOVO-Industri, Kopenhagen),
  - glucoamylase from *A. niger* (0.16 mL kg^{-1} FS, e.g., Optisprit-L, Solvay Enzymes, Hannover),
  - glucoamylase from *Rhizopus* sp. (0.4 g kg^{-1} FS, e.g., Optilase G 150, Solvay Enzymes, Hannover).
- A saccharification rest of 30 min is maintained at 52–53 °C.
- The mash is then cooled to 30 °C, and rinsed quantitatively into an Erlenmeyer flask.
- 1.0 g of a dried distillers', pure-culture yeast, which has been rehydrated before the test is added.
- The Erlenmeyer flask is then closed with a fermentation tube and placed into a water bath at 30 °C. The mash is fermented there for 3 d.

Autoamylolytical fermentation test without using any technical enzymes:

- 80.0 g of thoroughly milled grain are weighed in a mash beaker of a laboratory masher.
- 300 mL of H_2O are added.
- The pH is adjusted to 5.5.
- Each charge is dispersed for 1.5 min, using a laboratory dispersing machine (e.g., ULTRA TURRAX™).
- The mash beaker is placed in a laboratory masher and stirred.
- The laboratory masher is heated to the liquefaction temperature of 60 °C.
- While stirring, the charges are maintained at liquefaction temperature for 60 min.
- After the rest, the pH is adjusted to 5.0–5.2.
- Cooling to 30 °C and further processing is done as in the fermentation test using technical enzymes, except that no technical enzymes are used.

When the fermentation is completed, the mashes are distilled using a laboratory still, ethanol is measured by araeometry or densimetry, and the AAQ is calculated.

If the AAQ is higher than 95%, it is possible to process the raw material using one of the autoamylolytical processes described in Sect. 7. Fig. 24 shows the relation between ethanol yields from grains and the AAQ measured in these grains.

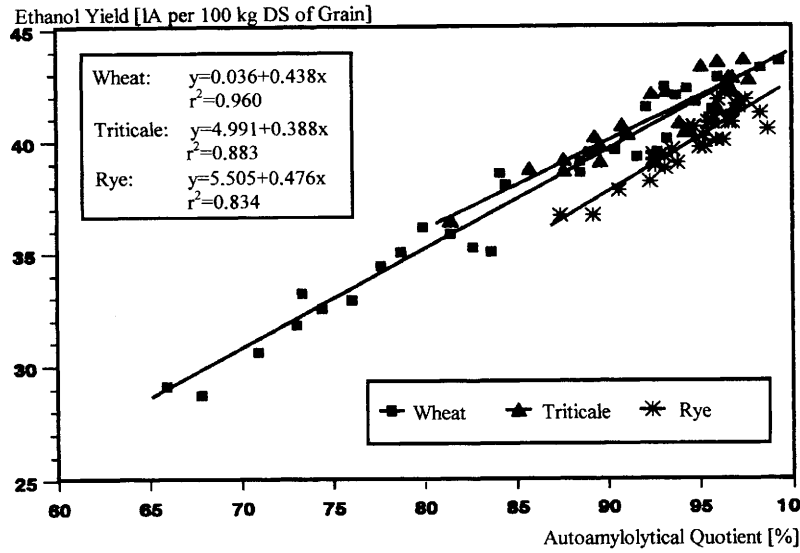

Fig. 24. Ethanol yields from wheat, triticale and rye: relation to autoamylolytical quotient.

13.3 Analysis of Mashes

13.3.1 Mash Hydrosizing

Mash hydrosizing which is an easy and effective method to examine the degree of decomposition of raw materials during the mashing process was developed by Pieper and Hotz (1988). The mash hydrosizer consists of a sediment cone fitted with two water nozzles to drain water into the cone. The outlets of the nozzles are aligned horizontally to create a rotating upward flow (Fig. 25). The flow rate of water drained into the hydrosizer depends on the raw material for the mashes (Tab. 24).

To examine a mash, water flow is started and 0.5 L of mash are poured into the sediment cone from the top. Then the water flow is adjusted exactly, and the mash is hydrosized for 10 min, during which time the solids are separated into three fractions:

Tab. 24. Flow Rates of Water Used in Hydrosizing of Mashes from Different Raw Materials

Raw Material	Flow Rate [L h^{-1}]
Potatoes	180
Wheat, rye, triticale	260
Barley	280
Corn	300

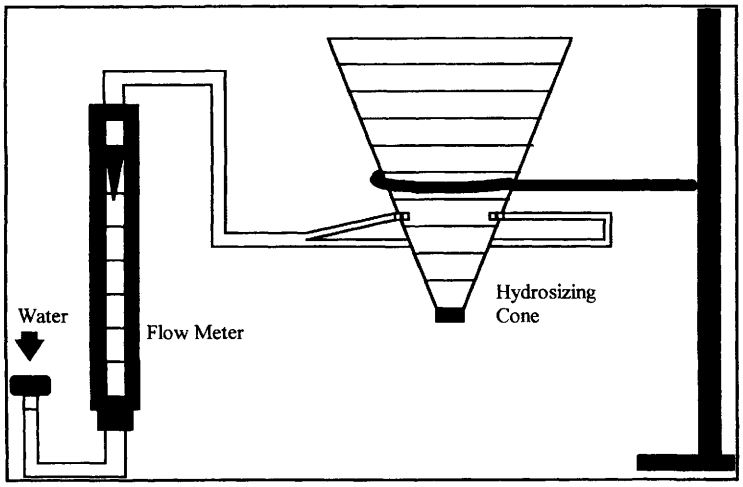

Fig. 25. Mash hydrosizer.

- the light fraction, which is rinsed out of the sediment cone on the top, containing small and light particles, already digested;
- the suspended fraction, remaining in the hydrosizer and containing only small amounts of starch that is not released from the cell material;
- the coarse fraction, which settles to the bottom of the sediment cone, even though water is still running; this fraction contains the heavy particles, that still contain large amounts of starch included in the cell material.

After 10 min the flow of water is stopped and the suspended fraction settles to the bottom of the hydrosizer. The sediment cone is scaled in mL, and so the amount of the coarse fraction and the sum of the suspended and coarse fractions are read off as results. To guarantee optimum ethanol yields from all starchy raw materials, hydrosizing should give the following maximum values:

Coarse fraction:	<1.5 mL
Coarse fraction + suspended fraction:	<5.0 mL
If barley is processed:	
Coarse fraction + suspended fraction:	<15.0 mL

13.3.2 Extract of Mashes

Only saccharometers according to Plato should be used to measure the extract content of mashes. These araeometers are scaled in %mas at 20 °C and are fitted with a thermometer and a temperature correction scale. It is necessary to

filter the mashes using folded filters before the extract content of mashes can be measured. The filtered mash is filled into a measuring cylinder, standing in a perpendicular position. The measured extract content represents the total content of solubilized substances, whether or not they are fermentable, and is not equivalent to the content of fermentable sugars. Therefore, the extract content depends not only on the starch content of the raw materials, but also on the mashing process.

The extract content in sweet mashes normally amounts to about 17%–18% mas using the HPCP. With pressureless mashing processes, the extract content amounts to 14%–16% mas if no stillage recycling is used. If stillage recycling is used, the extract content depends on the proportion of stillage recycling and amounts to 16%–20% mas in sweet mashes. The extract contents, measured when fementation is completed and when no stillage recycling is used in ethanol production, are shown in Tab. 14.

Using stillage recycling, the degree of fermentation depends on the proportion of stillage recycling used. However, using the same conditions and the same raw material in ethanol production results in the same degree of fermentation. So it is also possible to use measurements of the extract content and the degree of fermentation for the evaluation of fermentation efficiency.

13.3.3 pH of Mashes

The efficiency of the enzymatic treatment and the yeast fermentation depends greatly on the pH of mashes. Therefore, it is necessary to measure and to adjust the pH during the mashing processes and the fermentation depending on the enzymes used. The optimum pH values required for different processes and raw materials are described in Sects. 2 and 5.8. For the measurement of pH values only pH meters with a pH electrode should be used. The often used pH indicator paper is not precise enough, and the resulting color change depends not only on the pH value, but also on the color of the mashes.

13.3.4 Content of Ethanol in Mashes and Distillates

The ethanol content of mashes is determined by a laboratory distillation. The obtained distillate is measured using alcoholometers. In alcoholometry only alcoholometers authorized by the EU according to DIN 12803 should be used. These araeometers are calibrated to show the volume concentration of ethanol at 20 °C (% vol) in a mixture of ethanol and water. Additionally these alcoholometers are fitted with a thermometer. It is possible to measure ethanol concentrations in mixtures of ethanol and water between 0 °C and 30 °C by use of the official EU ethanol tables.

At the end of fermentation, mash solids start settling. A sample taken from a sampling cock is not an average sample. The best way to get reliable data is to filter the mashes, using folded filter papers, before distillation. Afterwards, 200 mL of mash filtrate are filled into the boiling flask of a laboratory still and diluted with 200 mL of H_2O; the pH is adjusted to 7.0. Then the diluted mash filtrate is distilled into a 200 mL one-mark volumetric flask, used as a receiving flask. The volumetric flask is removed from the lab still and the temperature is adjusted before it is filled up to the mark with distilled water. Then the ethanol concentration is measured using an EU-approved alcoholometer. The ethanol concentration measured in the laboratory distillate is equal to the ethanol content in mash filtrates. Therefore, the ethanol content in mash is calculated from the ethanol content determined in the mash filtrate times the spent grains factor. The grains factor is 0.97 for mashes from wheat, rye, triticale, corn, and potatoes, and 0.95 for barley mashes (Kreipe, 1981).

13.3.5 Microexamination

Microscopic examinations of mashes and yeast mashes should be carried out daily in a distillery. For this purpose, a microscope should be used that magnifies 1,000 times, preferably with phase-contrast. Microexamination is the only reliable method to determine whether the mashes are contaminated, and in addition shows if the yeast is in suitable condition.

It is not necessary to precisely count the microorganisms seen in the microscope; it is sufficient to classify the mashes as shown in Tab. 25. For this, a magnification of 1:1,000 is needed, and it is necessary to examine five or more different fields of view.

13.4 Analysis of Yeast Mashes

Yeast mash treatment is described in Sect. 9. It is important to carry out microexaminations and extract determinations daily and to watch the temperature and the pH value.

Tab. 25. Mash Classification Using Microexamination

Visual Examination	Contamination
No bacteria	O.K.
Single bacteria are visible	Technically free from contaminations
Number of bacteria up to 20% of yeast cells	Lightly contaminated
Up to number of bacteria = number of yest cells	Strongly contaminated
Number of bacteria > number of yeast cells	Cultivation of bacteria

In a yeast mash there may be no bacteria visible in microexaminations. The pH of the yeast mashes should be adjusted to 2.0 for about 2 h before the yeast mash is used for inoculation of sweet mash, if bacteria are present in yeast mashes. Otherwise, the pH of yeast mashes is adjusted to 2.5 (Sect. 9).

During fermentation of the yeast mash, the extract content should be reduced to about 50% of the extract content of the sweet mash, that was used in its preparation. The temperature of the yeast mash has to be kept below 27 °C until it is used for inoculation.

13.5 Analysis of Stillage

13.5.1 Content of Ethanol in Stillage

The stillage should be free of ethanol. If ethanol is present in stillage, this is due to a loss of ethanol and it shows that the distillation was not carried out with enough care. If ethanol is found in the stillage, usually the mash flow in the still is too high.

The determination of ethanol in stillage is carried out as described in Sect. 13.3.5. The stillage is not filtered before the sample is filled into the boiling flask of the laboratory still. Instead the samples of stillage have to be withdrawn directly from the bottom of the mash column. These samples must be filled immediately into a sample container that can be closed tightly. When the sample of stillage is cooled down in this tight container, it can be used for ethanol determination.

The ethanol contents measured in stillage are normally below 0.5% vol. It is not possible to get reliable data using a laboratory still, since stillage can not be compared with a mixture of ethanol and water; during the laboratory distillation of stillage, a lot of impurities (fatty acids, esters, aldehydes, etc.) are also distilled. This also occurs in the distillation of mashes, but the low ethanol content increases the error of measurement using araeometry or densimetry. Better results in the ethanol determined in stillage can be achieved if HPLC is used, as described in Sect. 13.5.2.

13.5.2 Content of Starch and Fermentable Sugars in Stillage

Normally the determination of residual starch and fermentable sugars in stillage is carried out using a fermentation test. The stillage is treated with thermostable α-amylases at 90 °C and with saccharifying enzymes at 55 °C as described in Sect. 13.2.3. After that, the stillage is fermented and distilled as described above, and the ethanol content is measured using alcoholometers. This method shows the same disadvantages as described in Sect. 13.5.1. Since, as a

rule, the content of FS in stillage amounts to not more than 2% mas, it is not sure if the FS is metabolized completetely or if the fermentation starts at all after the yeast is added.

Reliable results of tests for residual FS contents in stillage can be achieved if the HPLC method for the determination of FS in grain is used. For this, 300 g of stillage are weighed into the mash beaker, and there is no further dilution of stillage with this method. This is the only change in the method; further treatment is carried out as described in Sect. 13.2.2.1.

It is also possible to inject a sample of stillage already filtered, without further digestion, into the HPLC system, for the detection of ethanol and free fermentable sugars in the stillage. Using a Biorad column HPX-87-H, as described, some acids can also be detected. The HPLC method shows reliable results for residual contents of ethanol, free fermentable sugars and FS, and contamination during the fermentation. The FS determined in this way is a reliable indicator of the effectivity of the mashing process as well as of the fermentation and distillation, if stillage is examined once before and once after further digestion.

14 Energy Consumption and Energy Balance in Classical Processes

The energy consumption in ethanol production depends greatly on the process used. The energy used in some classical processes for ethanol production from potatoes, corn, and wheat is shown in Tabs. 26 and 27. The energy consumption also depends on the ratio of stillage recycling. Due to the fact that potatoes contain about 80–100% of the process liquid needed, processing of potatoes leads to the highest energy consumption in this comparison. It was found that DMP saves again 30% of energy consumption in the mashing process, com-

Tab. 26. Energy Consumption in Ethanol Production from Potatoes and Corn [MJ per hlA]

Raw Material	Potatoes	Corn	
Ratio of Stillage Recycling	15%	30%	50%
Mashing process			
Electrical energy	30	40	40
Thermal energy	380	170	150
Σ Mashing process	410	210	190
Distillation[a]	700	700	700
Total	1110	910	890

[a] 250 kg of steam per hlA = 700 MJ per hlA for distillation of raw spirit (85% vol.). These data depend strongly on the distillation equipment used.

Tab. 27. Energy Consumption in Ethanol Production from Wheat [MJ per hlA]

Raw Material	Wheat, DMP		HPCP[a]	Wheat[b]
Ratio of Stillage Recycling	30%	50%	–	–
Mashing process				
Electrical energy	40	40	20	60
Thermal energy	80	0	700	0
Σ Mashing process	120	40	720	60
Distillation[c]	700	700	700	700
Total	820	740	1420	760

[a] Pieper and Bohner (1985).
[b] Infusion process, using a spiral heat exchanger for heat recovery from stillage to mash (Goslich, 1990).
[c] 250 kg of steam per hlA = 700 MJ per hlA for a conventional distillation of raw spirit (85% vol.). These data depend strongly on the distillation equipment used.

pared with the process described by Goslich (1990). So it is possible to run a mashing process for ethanol production from wheat, rye, or triticale, consuming only 40 MJ per hlA, if DMP is used. This data were not just estimated but rather measured in practice, and it is reproducible in all plants using DMP.

If these data for energy consumption are compared with data from large-scale ethanol production (Misselhorn, 1980b), it can be seen that it is possible, using DMP and other classical methods, to produce ethanol from starchy raw materials with energy inputs that are lower than those calculated for large-scale plants (Tab. 28). Additionally one has to take into account than at a large-scale it is possible to produce dehydrated ethanol with the same energy that is needed at a small scale to produce raw spirit (85% vol).

Virtually all the energy balances for ethanol production from agricultural starchy raw materials are calculated with data from large-scale plants. Thus the

Tab. 28. Energy Consumption of Large-Scale Ethanol Production (Misselhorn, 1980b)

	Thermal Energy [MJ per hlA]	Electrical Energy [MJ per hlA]
Cleaning	2.2	7.2
Mashing process, batch	770	14.4
Mashing process, continuous	242	7.2
Fermentation	2.2	3.6
Distillation, conventional dehydrated ethanol	935	10.8
Distillation, multi-pressure system dehydrated ethanol	550	14.4
Total		
Batch process conventional distillation	1745	
Continuous process multi-pressure distillation	828	

Tab. 29. Energy Balance for Ethanol Production from Wheat or Triticale [GJ ha^{-1}] (Deutscher Bundestag, 1986 (FAL); Schäfer, 1995)

	FAL[a]	Triticale, DMP	Wheat, DMP
	Input		
Soil treatment		3.40	3.35
Fertilizers + biocides		11.00	11.00
Grain harvest		1.06	0.99
Straw pressing		1.70	1.54
Σ Agriculture	26.2	17.16	16.88
Mashing process		1.11	1.03
Fermentation + enzymes		0.56	0.51
Multipressure distillation		16.06	14.98
Σ Conversion	29.9	17.73	16.52
Σ Input	56.1		
With straw recovery		34.89	33.40
Without straw recovery		33.19	31.86
	Output		
Ethanol	44.5	58.69	54.76
Stillage	10.1	7.27	6.44
Straw	88.1	117.76	106.59
Biogas	9.6		
Σ Output	152.3	183.72	167.79
	Output : Input		
Bioethanol	0.96	1.77	1.72
Bioethanol + stillage	1.14	1.99	1.92
Bioethanol, stillage + straw	2.71	5.27	5.02

[a] Bundesforschungsanstalt für Landwirtschaft.

data given above is of great significance for energy balances. As shown in Tab. 29, energy data for DMP is of considerable importance in the energy balance of ethanol production. This energy balance (Schäfer, 1995; Loyce and Meynard, 1987) was calculated from empirically determined data for the growth of wheat and triticale in agriculture and from the conversion data derived from DMP. Compared with the data from large-scale plants (calculated), the ratio of energy output to input increases from 0.96 (FAL, see Tab. 29) to 1.77 (triticale, DMP). If the use of stillage as a fertilizer, and of straw (fuel value), is included in this calculation, the energy output to input ratio increases to 5.27.

Given these data on energy consumption and energy balance, it should be possible to produce bioethanol as a fuel component under much better conditions than currently thought. One also has to consider that if the stillage is used to produce biogas before it is used as a fertilizer, ethanol production is running energetically autarkical. In this manner it is possible to produce a fuel component within the CO_2 circle and surplus energy.

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Part II

The Biotechnology of Ethanol: Classical and Future Applications. Edited by M. Roehr

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Potential Source of Energy and Chemical Products

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1 Introduction

The environmental problems caused by the continued and increasing use of fossil feedstocks as energy source and the fact that the supply of many of their more desirable embodiments is approaching exhaustion are two main problems associated with the utilization of fossil fuels. Fermentative production of chemicals and energy production from renewable resources have been seriously considered as an alternative to petrochemical processes in recent years. Probably the best known of the alcohols which can be an alternative to petroleum is ethyl alcohol or ethanol. Billions of kilograms of it are produced annually. Ethyl alcohol is produced by chemical synthesis and by fermentation or biosynthetic processes. Pure ethanol is a colorless, limpid, volatile liquid which is flammable and toxic and has a burning taste. It boils at 78.4 °C and melts at −112.3 °C, has a specific gravity of 0.7851 at 20 °C, and is soluble in water and most organic liquids.

For well over one hundred years, researchers around the world have pursued ways to make ethanol from biomass such as wood, grasses, and waste materials. In general, ethanol made through fermentation is referred to as "bioethanol". The effort to develop bioethanol technology gained significant momentum in the late 1970s as a result of the energy crises that occurred in that decade. (Sheehan and Himmel, 1999).

Although ethanol is used as a solvent, extractant, antifreeze, as well as a fuel supplement, the major use of ethanol is as an intermediate feedstock in the synthesis of innumerable organic chemicals. Bimolecular dehydration of ethanol gives diethyl ether, which is employed as a solvent, extractant, and anesthetic. Dehydrogenation of ethanol yields acetaldehyde, which is the raw material for production of a large number of organic chemicals, such as acetic acid, acetic anhydride, chloral, butanol, crotonaldehyde, and ethylhexanol. Reaction with carboxylic acids or anhydrides yields esters which are useful in many applications. The hydroxyl group of ethanol may be replaced by halogen to give ethyl halides. Treatment with sulfuric acid gives ethyl hydrogen sulfate

and diethyl sulfate, a useful ethylating agent. Reaction of ethanol with aldehydes yields the respective diethyl acetals, and reaction with acetylene produces the acetals, as well as ethyl vinyl ether. These and other ethanol-derived chemicals are used in dyes, drugs, synthetic rubber, solvents, extractants, detergents, plasticizers, surface coatings, adhesives, moldings, cosmetics, explosives, pesticides, and synthetic fiber resins.

Ethanol, C_2H_5OH, is a chemical which is produced in largest volume by industrial fermentation. United States fuel ethanol production in 1998 exceeded the record production of 1.4 billion gallons set in 1995. Most of this ethanol was produced from over 550 million bushels of corn. Expanding fuel ethanol production will require developing lower-cost feedstocks, and only lignocellulosic feedstocks are available in sufficient quantities to substitute for corn starch. Major technical hurdles to converting lignocellulose to ethanol include the lack of low-cost efficient enzymes for saccharification of biomass to fermentable sugars and the development of microorganisms for the fermentation of these mixed sugars.

Rapid fermentation and high alcohol levels are desirable to minimize capital costs and distillation energy, while good yields are necessary for process economics (Llewellyn and Edwin, 1984). The efficiency of the production processes of ethanol depends on several aspects; operational simplicity, productivity and product concentration in the fermentation, and product recovery. In a fermentation, the productivity is proportional to the yeast cell concentration, and techniques to retain the cells in the fermentation section must ensure a high productivity (Groot et al., 1991). The concentration of cells in a bioreactor depends on the reactor design and on the biomass recycle ratio. High concentration of biomass in a reactor is possible when recycle bioreactors are used. In addition, product and substrate inhibition of the biomass determines productivity. Product inhibition also affects substrate consumption. High substrate and product levels inhibit cell growth, so productivity of the process is reduced. Temperature is another effective factor in fermentation since it affects the fermentation rate and also the cells' tolerance to high alcohol levels.

2 Microbiology and Biochemistry of Ethanol Formation

Industrially used microorganisms are selected to provide the best possible combination of characteristics for the process and equipment being used. The desired characteristics of an industrial ethanol process are highly dependent upon the choice of organism used in the fermentation. Organisms should have:

- a high yield of product per unit substrate assimilated,
- a high fermentation ability,

- substantial ethanol tolerance,
- the ability to remain viable at higher temperatures,
- stability under adequate fermentation conditions,
- a tolerance to low pH values.

Organisms must optimize these factors with respect to the process employed and the composition of the raw material. Yeasts and bacteria, which are widely used in industrial ethanol production, have several advantages and disadvantages.

To date, the most successful research approaches are focused on the development of novel biocatalysts, that will efficiently ferment mixed sugar syrups. Of particular interest is the isolation of novel yeasts that ferment xylose, genetic engineering of *Escherichia coli* and other gram-negative bacteria for ethanol production, and genetic engineering of *Saccharoymces cerevisiae* and *Zymomonas mobilis* for pentose utilization. Progress with new recombinant microorganisms has been rapid and will continue, leading to eventual development of organisms suitable for commercial ethanol production. Each research approach holds considerable promise, with the possibility that different "industrially hardened" strains may find separate applications in the fermentation of specific feedstocks.

Studies are also being conducted on the metabolic activities of ethanol-producing organisms in order to elucidate the mechanisms of product formation and increase productivity. Growth inhibition of microorganisms due to the accumulation of ethanol in the fermenter is one of the major problems that prevents time productivity in ethanol fermentation. Kobayashi et al. (1995) established an efficient ethanol production strategy by controlling culture temperature and ethanol concentration using a recycle system with a hollow fiber module. It was determined that, for efficient ethanol production, during the initial culture life the temperature should be maintained at approximately 30 °C and that ethanol concentration should be controlled at a low level to promote yeast growth. Furthermore, the draw-off through the filter should then be stopped and the temperature decreased from 30–20 °C. This new operational strategy attained a higher ethanol concentration and a higher ethanol productivity.

Moriyama and Shimizu (1996) suggested the application of a genetic algorithm (GA) for on-line determination of the optimal temperature profile for ethanol fermentation. The GA is defined as a probabilistic search method based on the mechanics of natural selection and natural genetics. The optimization was conducted by maximizing the final ethanol concentration with respect to culture temperature. The algorithm's effectiveness was examined by on-line measurement of ethanol and cell concentrations. A 14% increase in productivity was obtained by this method compared with the conventional constant-temperature fermentation.

Although the inherent complexity of the fermentation process means that complete prediction by means of a mathematical model is impossible, a model

is a useful tool for prediction of results, scaling up, and the design of experimental work. Garro et al. (1995) presented a mathematical model that may be defined as an unstructured and unsegregated model consisting of the microbial growth, rate of substrate consumption, and specific ethanol production rate kinetics. A triplicate batch fermentation using assay medium with different initial glucose concentrations was used to test the validity of the model. The predicted results were in good agreement with the experimental results.

2.1 Yeast Fermentation

The fermentation of sugar to ethanol by yeast has an important place among the different processes that are used in industry. The yeasts, which are of primary interest to industrial operations are *Saccharomyces cerevisiae*, *Saccharomyces uvarum (carlsbergensis)*, *Schizosaccharomyces pombe*, and *Kluyveromyces* species. The optimum conversion of sugar to ethanol requires a yeast strain capable of tolerating high ethanol concentrations, since ethanol inhibits growth and fermentation, although the latter is relatively more tolerant (del Castillo Agudo, 1985).

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

Glucose Ethanol Carbon dioxide

Yeasts are capable to utilize a variety of substrates (Tab. 1). In general, they are able to grow and efficiently ferment ethanol at pH values of 3.5–6.0 and temperatures of 28–35 °C. Though the initial rate of ethanol production is higher at increased temperatures (40 °C) the overall productivity of the fermentation is decreased due to ethanol product inhibition (Jones et al., 1981).

Yeasts, under anaerobic conditions, metabolize glucose to ethanol primarily by the Embden–Meyerhof pathway. The overall net reaction involves the production of 2 mol each of ethanol, CO_2, and ATP per mol of glucose fermented. Therefore, on a weight basis, each gram of glucose can theoretically give rise to 0.51 g alcohol. The yield attained in practical fermentations, however, does not usually exceed 90–95% of the theoretical value. This is due to the requirement for some nutrients to be utilized in the synthesis of new biomass and other cell maintenance-related reactions. Side reactions also occur in the fermentation (usually to glycerol and succinate) which may consume up to 4–5% of the total substrate. If these reactions could be eliminated, an additional 2.7% yield of ethanol from carbohydrate would result (Oura, 1977).

Fig. 1 represents a simplified scheme for the anaerobic and aerobic catabolism of *S. cerevisiae*. The Embden–Meyerhof–Parnas pathway for anaerobic metabolism of glucose to ethanol is shown in Fig. 2. The individual reactions and thermodynamics of glycolysis and alcoholic fermentation are shown on Tab. 2.

Tab. 1. The Ability of Saccharomyces and Kluyveromyces Species to Ferment Sugars (Jones et al., 1981)[a]

Carbon Number of Basic Subunit	Type of Basic Subunit	Sugar	Basic Unit	Yeast		
				S. cerevisiae	*S. uvarum (carlsbergensis)*	*Kluyveromyces fragilis*
6	aldoses	glucose	glucose	+	+	+
		maltose	glucose	+	+	−
		maltotriose	glucose	+	+	−
		cellobiose	glucose	−	−	−
		trehalose	glucose	+/−	+/−	−
		galactose	galactose	+	+	+
		mannose	mannose	+	+	+
		lactose	glucose, galactose	−	−	+
		melibiose	glucose, galactose	−	+	
	ketoses	fructose	fructose	+	+	+
		sorbose	sorbose	−	−	−
	aldoses and ketoses	sucrose	glucose, fructose	+	+	+
		raffinose	glucose, fructose galactose	+/−	+	+/−
	deoxy-sugars	rhamnose	6-deoxymannose	−	−	−
		deoxyribose	2-deoxyribose	+/−	+/−	+/−
5	aldoses	arabinose	arabinose	−	−	−
		xylose	xylose	−	−	−

[a] New taxonomy: *S. uvarum* included in *S. cerevisiae*, *K. fragilis* changed to *K. marxianus*.

A small concentration of oxygen must be provided to the fermenting yeast as it is a necessary component in the biosynthesis of polyunsaturated fats and lipids. Typical amounts of O_2 to be maintained in the broth are 0.05–0.10 mm Hg oxygen tension. Any values higher than this will promote cell growth at the expense of ethanol productivity (i.e., the Pasteur effect).

The relative requirements for nutrients not utilized in ethanol synthesis are in proportion to the major components of the yeast cell. These include carbon, oxygen, nitrogen, and hydrogen. Small quantities of phosphorus, sulfur, potassium, and magnesium must also be provided. Minerals (i.e., Mn, Co, Cu, Zn) and organic growth factors (amino acids, nucleic acids, and vitamins) are required in trace amounts. The most important growth factors for yeast are the vitamins, biotin, pantothenic acid, inositol, thiamine, nicotinic acid, and folic acid (Maiorella et al., 1981).

Ergun et al. (1997) investigated the influence of ethylenediamine tetraacetic acid, potassium ferrocyanide, and zeolite X on ethanol production from sugar beet molasses by *S. cerevisiae*. A more pronounced effect was observed on

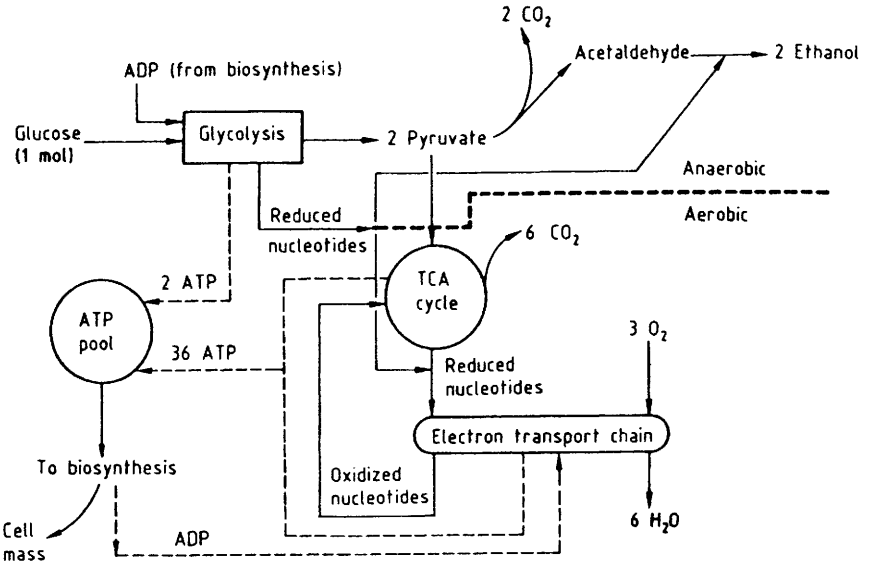

Fig. 1. Simplified chart of anaerobic and aerobic catabolism of *Saccharomyces cerevisiae* ADP: adenosine diphosphate; ATP: adenosine triphosphate; TCA: tricarboxylic acid (citric acid).

addition of each of the substances to the fermentation medium rather than to the growth medium. The greatest stimulation in product yield was achieved with the introduction of zeolite X during the fermentation stage.

Many feedstocks under consideration for large-scale ethanol production supply all nutrients necessary for yeast growth in addition to carbohydrate for bioconversion. Additional supplementation with nutrients may be required in some cases. These nutrients may be provided as individual components such as ammonium salts and potassium phosphate or from a low-cost source such as corn steep liquor.

Yeasts are very susceptible to ethanol inhibition. Concentrations of 1–2% (w/v) are sufficient to retard microbial growth and at 10% (w/v) alcohol, the growth rate of the organisms is nearly halted (Brown et al., 1981).

Over long fermentation times, ethyl alcohol exhibits traditional non-competitive Michaelis–Menten inhibition on microbial growth (Aiba et al., 1968), however, studies by Brown et al. (1981) indicate that the immediate effects of this inhibition are more complex. Addition of ethanol to log phase yeast cultures results in a rapid reduction of growth rate (possibly due to effects on protein synthesis), a decrease in cell viability (through irreversible denaturation of enzymes), and to a much lesser extent ethanol lowers the rate of its own synthesis. Observations that the extent of ethanol tolerance for certain yeast strains is dependent upon the fatty acyl composition of their plasma mem-

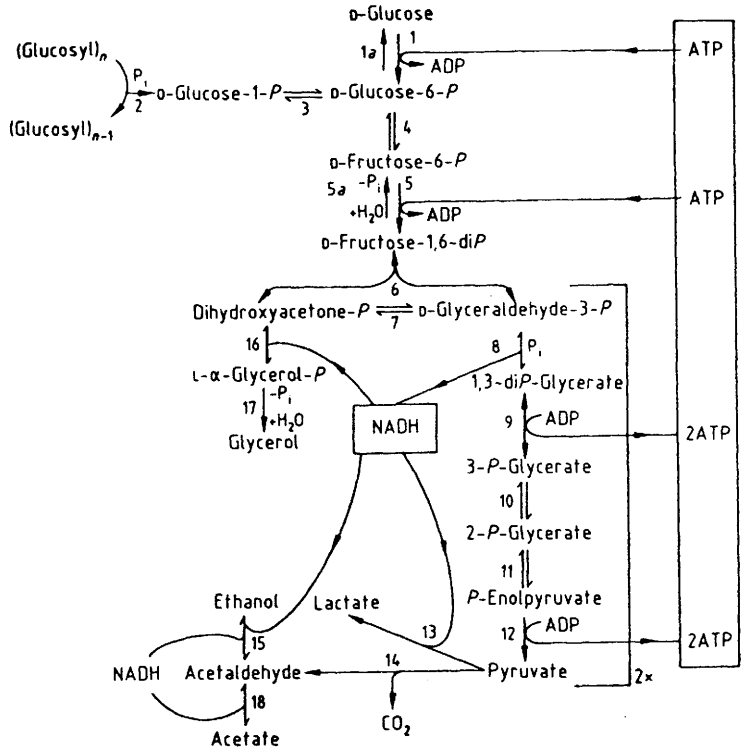

Fig. 2. Embden–Meyerhof–Parnas scheme of glycolysis.
Glycolysis:
Glucose + 2 P_i + 2 ADP → 2 Lactate + 2 ATP (classical mammalian muscle or brain)
Glucose + P_i → α-Glycerol phosphate + pyruvate (insect flight muscle, striated muscle)
Fermentation:
Glucose + 2 P_i + 2 ADP → 2 Ethanol + 2 CO_2 + 2 ATP + 2 H_2O (1st form)
Glucose + HSO_3^- → Glycerol + Acetaldehyde · HSO_3 + CO_2 (2nd form, no net ATP)
Glucose + (P_i) → α-Glycerol phosphate + Acetaldehyde + CO_2
↓
Glycerol + Pi (3rd form, no net ATP)

branes (Thomas and Rose, 1979) would indicate that the fatty acyl composition favors or inhibits excretion of ethanol from the plasma.

In Tab. 3 and Fig. 3 some of the kinetic models and inhibition constants are compared which have been proposed to quantify the effects of ethanol inhibition. As can be seen, there is a great variability between these models. It has been demonstrated (Novak et al., 1981; Hoppe and Hansford, 1982) that ethanol which is produced during the fermentation (autogenous ethanol) is more inhibitory to cell growth than that added artificially from an exogenous source. Apparently exogenously added ethanol cannot build up the respective intracellular concentration required for inhibition.

Tab. 2. Reactions and Thermodynamics of Glycolysis and Alcoholic Fermentation

Reaction Number	Equation[a]	Name of Enzyme	Characteristic Inhibitor	$\Delta G^{0'b}$, [kJ mol^{-1}]
1	Glucose + ATP $\xrightarrow{Mg^{2+}}$ Glucose 6-*P* + ADP	hexokinase glucokinase		−14.32
1a	Glucose 6-P + H_2O $\xrightarrow{Mg^{2+}}$ Glucose + P_i	glucose 6-phosphatase nonspecific phosphatases	glucose, orinase	−16.83
2	Glycogen + *n* P_i ⇌ *n* Glucose 1-*P*	α-1,4-glucan phosphorylase		3.06
3	Glucose 1-*P* $\rightleftharpoons$ Glucose 6-*P*	phosphoglucomutase	F, organophosphorus inhibitors	− 7.29
4	Glucose 6-*P* ⇌ Fructose 6-*P*	phosphoglucose (glucose phosphate) isomerase	2-deoxyglucose 6-phosphate	2.09
5	Fructose 6-P + ATP $\xrightarrow[(ADP, AMP)\ K^+]{Mg^{2+}}$ Fructose 1,6-di*P* + ADP + H+	phosphofructokinase	ATP, citrate	−14.24
5a	Fructose 1,6-di*P* + H_2O $\xrightarrow{Mg^{2+}}$ Fructose 6-*P* + P_i	fructose diphosphatase nonspecific phosphatases	AMP, fructose 1,6-diphosphate, Zn^{2+}, Fe^{2+}	−16.75
6	Fructose 1,6-di*P* ⇌ Dihydroxyacetone *P* + Glyceraldehyde 3-P	(fructose phosphate) aldolase	chelating agents (microbial enzymes only)	23.99
7	Dihydroxyacetone P ⇌ Glyceraldehyde 3-*P*	triose phosphate isomerase		7.66
8	2 × (Glyceraldehyde 3-*P* + P_i + NAD^+ ⇌ 1,3-Diphosphoglycerate + NADH + H^+)	glyceraldehyde phosphate dehydrogenase; triose phosphate dehydrogenase	ICH_2COR D-threose 2,4-diphosphate	2 × (6.28)
9	2 × p(1,3-Diphosphoglycerate + ADP + H^+ $\xrightarrow{Mg^{2+}}$ Phosphoglycerate + ATP)	phosphoglycerate kinase		2 × (−28.39)

Tab. 2. Reactions and Thermodynamics of Glycolysis and Alcoholic Fermentation (Continued)

Reaction Number	Equation[a]	Name of Enzyme	Characteristic Inhibitor	$\Delta G^{0'}$[b], [kJ mol^{-1}]
10	$2\times$(3-Phosphoglycerate $\overset{\text{Glycerate 2,3-di}P}{\rightleftharpoons}$ 2-Phosphoglycerate)	phosphoglyceromutase		$2\times(4.43)$
11	$2\times$(2-Phosphoglycerate $\overset{Mg^{2+}\text{ or }Mn^{2+}}{\rightleftharpoons}$ Phosphoenolpyruvate)	enolase (phosphopyruvate hydratase)	Ca^{2+} F^- plus P_i	$2\times(1.84)$
12	$2\times$(Phosphoenolpyruvate + ADP + H + $\xrightarrow[K^+(Rb^+, Cs^+)]{Mg^{2+}}$ Pyruvate + ATP)	pyruvate kinase	Ca^{2+}, vs. Mg^{2+} Na^+ vs. K^+	$2\times(-23.95)$
13	$2\times$(Pyruvate + NADH + H^+ $\rightleftharpoons$ Lactate + NAD^+)	lactate dehydrogenase	oxamate	$2\times(-25.12)$
14	$2\times$(Pyruvate + H^+ $\rightarrow$ Acetaldehyde + CO_2)	pyruvate (de)carboxylase		$2\times(-19.76)$
15	$2\times$(Acetaldehyde + NADH + H^+ $\rightleftharpoons$ Ethanol + NAD^+)	alcohol dehydrogenase	(HSO_3^-)	$2\pm(-21.56)$
Sums:	$(Glucose)_n + H_2O \rightarrow 2$ Lactate $+ 2\ H^+ + (Glucose)_{n+1}$			-219.40
	$(Glucose)_n + 3\ P_i + 3$ ADP $\rightarrow 2$ Lactate $+ 3$ ATP $+ (Glucose)_{n-1}$		glycolysis (muscle)	-114.38[c]
	Glucose $\rightarrow 2$ Lactate $+ 2$ H +			-198.45
	Glucose $+ 2\ P_i + 2$ ADP $\rightarrow 2$ Lactate $+ 2$ ATP		glycolysis or lactate fermentation	-124.52[c]
	Glucose $\rightarrow 2$ Ethanol $+ CO_2$			-234.88
	Glucose $+ 2\ P_i + 2$ ADP $\rightarrow 2$ Ethanol $+ 2\ CO_2 + 2$ ATP		alcoholic fermentation	-156.92[c]

[a] Cosubstrates or coenzymes shown above, activators below arrow; P: phosphate.
[b] $\Delta G^{0'}$ values refer to pH 7.0 with all other reactants including H_2O at unit activity; the free energy of formation of glucose in aqueous solution equals 910.88 kJ mol^{-1}, its ΔG^0 of combustion to $CO_2 + H_2O$ is 2872 kJ mol^{-1}, and $\Delta G^{0'}$ for $(glucose)_n + H_2O \rightarrow (glucose)_{n-1}$ equals -21.06 kJ mol^{-1}.
[c] From this table.

Tab. 3. Inhibition Kinetic Constants Associated with Data Procuded by Different Authors (Hoppe and Hansford, 1982)

Parameter	Added Ethanol			Autogenous Ethanol		
	Egamberdiev and Ierusalimskii (1968)	Aiba and Shoda (1969)	Bazua and Wilke (1977)	Pironti (1971)	Cysewski (1976)	Hoppe and Hansford (1982)
°C	28	30	35	30	35	30
μ_{max} $[h^{-1}]^a$	0.31	0.43	0.64	0.26	0.58	0.64
K_s $[g\,L^{-1}]^b$	–	–	0.24	15.5	4.9	3.3
K_p $[g\,L^{-1}]^c$	20.6	55	40	13.7	5.0	5.2
$Y_{p/s}{}^d$	0.39	0.35	0.52	0.47	0.44	0.43

[a] Maximum specific growth rate
[b] Substrate saturation constant
[c] Product inhibition constant
[d] Product yield coefficient

The main advantage of *S. cerevisiae* is that it can utilize a variety of substrates such as glucose, maltose, galactose, fructose, and sucrose. In general, yeasts are able to grow and efficiently produce ethanol at pH values between pH 3.5 and 6.0 and temperatures from 28 to 35 °C. So contamination is a small-

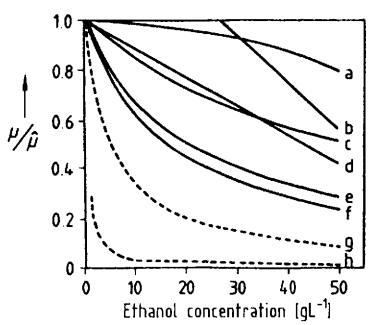

Fig. 3. Comparison of the effect of various ethanol inhibition functions (Hoppe and Hansford, 1982).
(a) Bazua and Wilke (1977), continuous,
(b) Holzberg et al. (1967), batch
(c) Aiba and Shoda (1969), continuous
(d) Ghose and Tyagi (1979), continuous
(e) Egamberdiev and Ierusalimskii (1968), batch
(f) Aiba and Shoda (1969), batch
(g) Hoppe (1981), continuous
(h) Strehaiano et al. (1978), batch
μ: specific growth rate; $\hat{\mu}$: maximum specific growth rate; source of ethanol: ——— added, – – – – autogenous.

er threat with decrease in pH from pH 6.3 to 3.3 during the fermentation. However, though the initial rates of ethanol production are higher at increased temperatures such as 40 °C, the overall productivity of the fermentation is decreased due to enhanced ethanol inhibition (Kosaric, 1984) and this is a disadvantage of *S. cerevisiae*.

2.2 Ethanol Fermentation with Bacteria

A great number of bacteria are capable of ethanol formation (Buchanan and Gibbons, 1974). Many of these microorganisms, however, generate multiple end products in addition to ethyl alcohol. These include other alcohols (butanol, isopropyl alcohol, 2,3-butanediol), organic acids (acetic, butyric, formic, and lactic acid), polyols (arabitol, glycerol, and xylitol), ketones (acetone), or various gases (methane, carbon dioxide, hydrogen). The microorganisms which are capable of producing ethanol as the major product (i.e., a minimum of 1 mol ethanol produced per mol of glucose utilized) are shown in Tab. 4.

Tab. 4. Bacterial Species Producing Ethanol as the Main Fermentation Product (Wiegel, 1980)

Mesophilic Organisms		mmol Ethanol Produced per mmol Glucose Metabolized
Clostridum sporogenes		up to 4.15[a]
Clostridium indolis (*pathogenic*)		1.96[a]
Clostridium sphenoides		1.8[a] (1.8)[b]
Clostridium sordelli (*pathogenic*)		1.7
Zymomonas mobilis (syn. *anaerobica*)		1.9 (anaerobe)
Zymomonas mobilis ssp. *pomaceae*		1.7
Spirochaeta aurantia		1.5 (0.8)
Spirochaeta stenostrepta		0.84 (1.46)
Spirochaeta litoralis		1.1 (1.4)
Erwinia amylovora		1.2
Leuconostoc mesenteroides		1.1
Streptococcus lactis		1.0
Sarcina ventriculi (syn. *Zymosarcina*)		1.0
Thermophilic Organisms	T_{max} [°C]	mmol Ethanol Produced per mmol Glucose Utilized
Thermoanaerobacter ethanolicus (gen. nov.)	78	1.9
Clostridum thermohydrosulfuricum	78	1.6
Bacillus stearothermophilus	78	1.0 (anaerobic above 55 °C)
Thermoanaerobium brockii	78	0.95
Clostridium thermosaccharolyticum (syn. *tartarivorum*)	68	1.1
Clostridium thermocellum (*thermocellulaseum*)	68	1.0

[a] In the presence of high amounts of yeast extract.
[b] Values in brackets were obtained with resting cells.

Many bacteria (i.e., Enterobacteriaceae, *Spirochaeta*, *Bacteroides*, etc.), as yeasts, metabolize glucose by the Embden–Meyerhof pathway. The Entner–Doudoroff pathway is an additional means of glucose consumption in many bacteria. Glucose is phosphorylated and then oxidized to 6-phosphogluconate. At this point, dehydration occurs to form 2-keto-3-deoxy-6-phosphogluconate (KDPG) which is then cleaved by KDPG-aldolase. The net yield is 2 mol pyruvate formed from 1 mol glucose and the generation of 1 mol ATP.

Multiple end products may be produced by organisms which conduct mixed acid-type fermentations such as the "enteric" group of facultative anaerobic bacteria. The possible routes of these complex pathways are illustrated in Fig 4. Phosphoenolpyruvate produced in the Embden–Meyerhof pathway may be further broken down to such diverse products as ethanol, formate, acetate, succinate, lactate, and 2,3-butanediol.

As can be seen from Tab. 4, a number of bacteria are able to produce relatively high yields of ethanol. Although some mesophilic *Clostridium* strains are capable of yielding higher concentrations, only *Zymomonas mobilis* can be regarded as a strict ethanol producer.

Zymomonas mobilis have been shown to give a very high product concentration without inhibition (Maiorella et al., 1981). In addition, ethanol production under aerobiosis is also possible since *Z. mobilis* exhibits no Pasteur effect. However, the choice of substrates is limiting for ethanol production. Al-

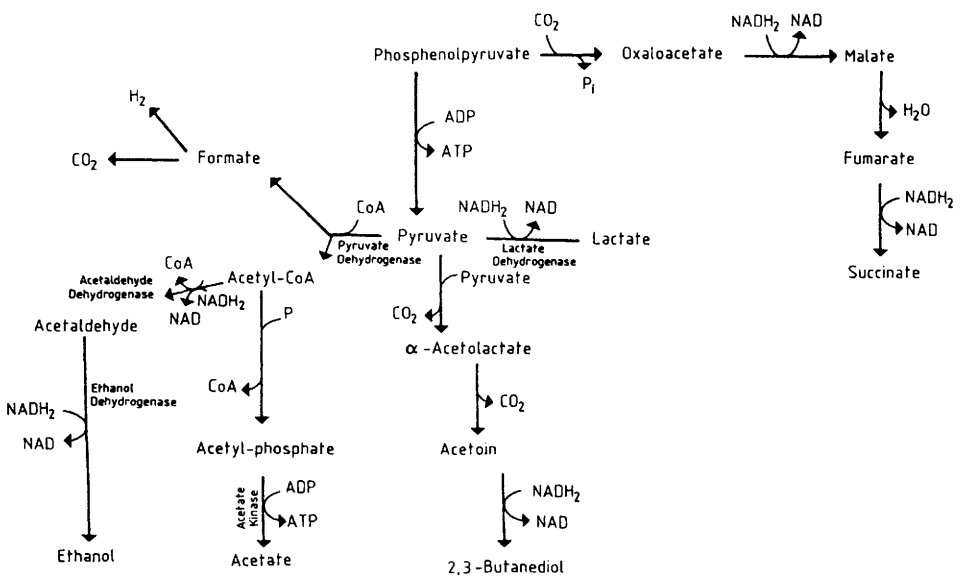

Fig. 4. Pathways for the formation of products of the mixed acid and 2,3-butanediol fermentations (Rosenberg, 1980)

though all of the strains of this bacterium can use glucose, only 50% of them can convert sucrose to glucose. The usage of sucrose causes excessive quantities of unwanted by-products during the fermentation and this is a disadvantage of *Z. mobilis*. The strong production of organic acids (e.g., acetic acid, lactic acid) which cause problems during the rectification of the ethanol is another important disadvantage of the utilization of *Z. mobilis* in ethanol production. The optimum temperatures and pH values found by the experiments are 30–40 °C and pH 4.0–5.0, respectively, for this bacterium. Since the decrease in pH value is small, the medium must be sterilized when this organism is used.

Lee et al. (1981) studied the fermentation kinetics of *Z. mobilis* ZM4 on artificial media containing either glucose, fructose, or sucrose as carbon source. Kinetic data for growth of this bacterium are presented in Tab. 5. It is apparent that the specific rates of sugar uptake and ethanol production are at a maximum when utilizing the glucose medium. Major differences are observed in cell yield with decreased values for growth in fructose and sucrose medium. Substrate inhibition of this bacterial species is not severe as it has been shown and growth will occur up to a glucose concentration of 40% (w/v) (Swings and DeLey, 1977).

Continuous cultivation of *Z. mobilis* on glucose media has been investigated by Rogers et al. (1980). Their results are presented in Fig. 5. With a glucose feed concentration of 100 g L^{-1}, stable growth was achieved with ethanol concentrations up to 49 g L^{-1}. Complete utilization of a 100 g L^{-1} glucose solution was achieved at a dilution rate of 2.0 h^{-1} in a *Z. mobilis* bioreactor employing cell recycle by means of membrane filtration (Rogers et al., 1980). Volumetric ethanol productivity was reported to be 120 g L^{-1} h^{-1} with a steady state ethanol concentration of 48 g L^{-1}.

Tab. 5. Kinetic Parameters for Growth of Zymomonas mobilis Strain ZM4 in Batch Culture with Different Carbon Substrates (Rosenberg, 1980)

Kinetic Parameter	Substrate		
	Glucose	Fructose	Sucrose
Specific growth rate, μ [h^{-1}]	0.18	0.10	0.14
Specific substrate consumption rate, q_s [g g^{-1} h^{-1}]	11.3	10.4	10.0[a]
Specific ethanol production rate, q_s [g g^{-1} h^{-1}]	5.4	5.1	4.6
Cell yield, Y [g g^{-1}]	0.015	0.009	0.0014
Ethanol yield, $Y_{P/S}$ [g g^{-1}]	0.48	0.48	0.46
Ethanol yield [% of theoretical]	94.1	94.1	90.2[b]
Maximum ethanol concentration [g L^{-1}]	117	119	89
Time period of calculation of maximum rates [h]	0–19	0–28	0–15

[a] Based on changes in total reducing sugar after inversion.
[b] Not corrected for levan formation.

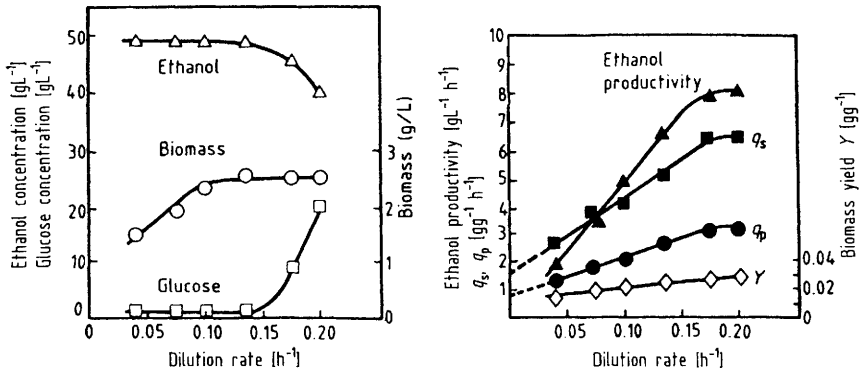

Fig. 5. Steady-state data and kinetic parameters for growth of *Zymomonas mobilis* in continuous culture on 10% glucose medium (Rogers et al., 1980).

2.2.1 Thermophilic Organisms

The selected strains normally operate best at pH 4.5 and 28–30 °C, with efficiency dropping off rapidly at higher temperatures (Keim, 1983). However, the substrate must be cooled since the fermentation is exothermic. The advantages of using thermophilic microbes in the production of fuel alcohol are numerous. Since ethanol-producing yeasts growing at an optimal fermentation temperature of 50 °C have to be discovered yet, it is apparent that thermophilic bacteria (Tab. 4) are necessary for these processes.

According to Wiegel (1980) some benefits of high-temperature fermentation are as follows:

- Thermophiles exhibit high catabolic activity at the temperatures optimal for their growth. This results in shorter fermentation times, higher productivity, and an overall increase in the efficiency of fermentation.
- The solubility of oxygen and other gases in the fermentation broth decreases with increasing temperature. This phenomenon supports the establishment and long-term maintenance of anaerobic conditions. The optimum temperature of extreme thermophiles, e.g., is 66–69 °C and at this temperature, the solubility of O_2 in the media is 80% lower than at 30 °C.
- Substrates with low solubilities at ambient temperatures would exhibit greater solubility at optimal fermentation temperatures. As such, it is possible that substrate availability would no longer be the rate-limiting step for the process.
- The viscosity of the fermentation broth decreases with increasing temperature. Therefore, the energy required to maintain proper agitation of the growth media is lowered.

- The recovery of ethanol is enhanced at high temperatures. This fact may be utilized in combination with such continuous ethanol removal processes as Vacuferm. The increase of alcohol in the gaseous phase decreases the required degree of vacuum necessary for efficient product recovery.
- The metabolic activity of microbes and frictional effects of agitation serve to generate large amounts of heat. Thus, additional energy to maintain the vessels at the desired temperature as well as the cooling requirements after sterilization are minimized.
- Compared to mesophilic bacteria, sterile conditions are not as essential in a thermophilic process. No obligate thermophilic pathogens are known at present. However, contamination may occur by thermophilic fungi and other bacteria.

Interest has also been centered upon the extreme thermophilic chemoorganotrophic anaerobe, *Thermoanaerobacter ethanolicus* (Wiegel and Ljungdahl, 1981). In addition to its extreme thermophilic nature (temperature optimum 69 °C), *T. ethanolicus* has two basic advantages over other organisms:

(1) It exhibits a very broad pH optimum of 5.5–8.5 (growth will occur at pH 4.5–9.5).
(2) It is able to ferment carbohydrates to an almost quantitative yield. *T. ethanolicus* is capable of utilizing a wide range of substrates to produce ethanol. These include starch, cellobiose, lactose, and various pentose sugars. Relative merits of mesophilic and thermophilic fermentation for alcohol production were also discussed by Mistry (1991).

2.3 Bacteria vs. Yeast

Comparison of fermentations conducted by yeast and bacteria can be made on the basis of the kinetics of both systems. The differences observed reflect the metabolic dissimilarities in these groups of organisms.

From Tab. 6 it can be seen that for batch growth at the conditions indicated, *Zymomonas mobilis* conducts a more efficient fermentation than *Saccharomyces uvarum*. Key kinetic parameters indicating the superiority of this bacterial species include specific growth rate, μ (2.4 times higher than yeast), specific ethanol production rate, q_p (2.9-fold increase), and specific glucose uptake rate q_s (2.6 times higher than for *S. uvarum*).

For maximum ethanol productivity in continuous systems, bacteria are superior to yeasts. The greatest productivity achieved by a yeast system (cell recycle with vacuum) was only 68% of that reported for a *Z. mobilis* process employing cell recycle (Tab. 7) (Rogers et al., 1980). The data presented in Tab. 7 indicate that for cell recycle systems operating at the same glucose input concentration (100 g L^{-1}), bacterial ethanol productivity is 4.1 times that of yeast.

Tab. 6. Kinetic Parameters for Zymomonas mobilis and Saccharomyces uvarum on 250 g L^{-1} Glucose Media in Non-Aerated Batch Culture (30 °C, pH 5.0) (Rogers et al., 1980)

Kinetic Parameter	*Z. mobilis*	*S. uvarum*
Specific growth rate, μ [h^{-1}]	0.13	0.055[c]
Specific glucose uptake rate, q_s [g g^{-1} h^{-1}]	5.5	2.1c
Specific ethanol production rate, q_p [g g^{-1} h^{-1}]	2.5	0.87[c]
Cell yield, Y [g g^{-1}][a]	0.019	0.033
Ethanol yield, $Y_{p/s}$ [g g^{-1}][a]	0.47	0.44
Relative ethanol yield [%][a, b]	92.5	86
Maximal ethanol concentration [g L^{-1}]	102	108

[a] Based on the difference between initial and residual glucose concentrations.
[b] A molar reaction stoichiometry of 1 Glucose → 2 Ethanol + 2 CO_2 has been assumed for a theoretical yield.
[c] Kinetic parameters calculated for a fermentation run between 16 and 22 h, the culture growing fully anaerobically.

Tab. 7. Comparative Ethanol Productivities of Continuous Systems (Rogers et al., 1980)

Organism	System	Optimal Growth Conditions				Maximal Productivity
		Input Glucose [g L^{-1}]	Dilution Rate [h^{-1}]	Cell Concentration [g L^{-1}]	Ethanol Concentration [g L^{-1}]	[g L^{-1} h^{-1}]
Saccharomyces cerevisiae ATCC 4126	no recycle	100	0.17	12	41	7.0
S. cerevisiae ATCC 4126	cell recycle	100	0.08	50	43	29
S. cerevisiae NRRL Y-132	cell recycle	150	0.53	48	60.5	32
S. uvarum ATCC 26602	cell recycle	200	0.60	50	60	36
S. cerevisiae ATCC 4126	vacuum (6.7 kPa)	334		50	100–160[b]	40
S. cerevisiae ATCC 4126	cell recycle Vacuum (6.7 kPa)	334	0.23[a]	124	110–160[b]	82
Zymomonas mobilis ATCC 10988	cell recycle	100	2.7	38	44.5	120

[a] Based on bleed rate from clarifier.
[b] Concentration in vapor stream from vacuum fermenter.

In yeast fermentations oxygen is required for cell wall synthesis, stabilization of lipid structures, and general maintenance of cellular processes. However, aerobic conditions also lead to a decrease in ethanol yield and a subsequent increase in biomass concentration due to the Pasteur effect. Since many bacteria

are strict anaerobes, with them higher ethanol productivities and lower biomass production are possible. Lower bacterial cell concentration is also a consequence of the reduced energy available for growth under anaerobic conditions (1 mol ATP per mol glucose consumed via the Entner–Doudoroff pathway vs. 2 mol ATP via the Embden–Meyerhof pathway using yeast).

Data have been reported (Rogers et al., 1980), which show that the ethanol tolerance of *Z. mobilis* is equivalent or higher than that of certain strains of *S. cerevisiae*. It is necessary to specify the carbon source which the organism utilizes before comparisons of this type can be made.

Qureshi and Manderson (1995) considered various fermentation technologies, including continuous culture and cell recycle. Ethanol recovery was examined using pervaporation and costs compared with distillation. The effects on ethanol prices of raw material costs, fermentation technology, product recovery, tax, plant size, and Lang factor are presented. Cultures of *Candida shehatae*, *Zymomonas mobilis*, *Kluyveromyces marxianus* var. *lactis*, and *Saccharomyces cerevisiae* (with *Zymomonas mobilis*) were used, depending on the substrate. The most appropriate technologies in terms of final ethanol price were reported.

The question which organism, *Zymomonas mobilis* or *Saccharomyces cerevisiae*, is more suitable for industrial fermentation has to be decided on the basis of ease of handling. The high yield of ethanol and the low yield of biomass of *Z. mobilis* is counter balanced by the necessity to sterilize the culture medium. Thus, from an economic point of view *S. cerevisiae* is generally preferred.

2.4 Genetically Modified Organisms

Future progress in ethanol tolerance and the range of possible substrates used in these fermentation systems may be enhanced through genetic recombination techniques. Although a multitude of strain variations from these yeasts have been produced through genetic manipulations, due to their prokaryotic nature, manipulation of the genetic material in bacterial species is carried out with greater ease than in yeasts. Today, through genetic engineering one can obtain more versatile bacteria that can produce ethanol from cheaper feedstocks and considerably reduce the cost of producing ethanol from about $1.20 per gallon. The Department of Energy hopes that ethanol's wholesale price will be $0.90 per gallon by 2010 and $0.60–0.50 per gallon by 2020 (Ondrey and Armesto, 1999).

The natural form of *Zymomonas mobilis* produces ethanol from the 6-carbon sugars glucose and fructose and the disaccharide sucrose. In 1993, in the United States, at the University of Florida, Ingram reported a development of series of genetically engineered bacteria which efficiently ferment all of the sugars present in lignocellulose (Ingram, 1993). This was done by inserting a

portable, artificial operon containing the *Z. mobilis* genes for alcohol dehydrogenase and pyruvate decarboxylase into other bacteria with a native ability to metabolize different sugars. Organisms have been developed which can ferment cellobiose, cellotriose, xylobiose, xylotriose, maltose, maltotriose, and other oligomeric sugars. The depolymerization of monomeric sugars prior to fermentation was not required. Efficiencies exceeded 90% of theoretical yields. Researchers at the Department of Energy's National Renewable Energy Laboratory in Golden, Colorado, have genetically modified the bacterium *Z. mobilis*, so that it also makes ethanol from the 5-carbon sugar xylose. Although naturally occurring *Z. mobilis* makes ethanol in 5 to 10% higher yield than does yeast, it has not yet been applied commercially (Borman, 1995).

Various strains have been developed and evaluated by Bothast et al. (1999). *E. coli* KO11, *E. coli* SL40, *E. coli* FBR3, *Zymomonas* CP4 (pZB5), and *Saccharomyces* 1400 (pLNH32) fermented corn fiber hydrolyzates to ethanol in the range of 21–34 g L^{-1} with yields ranging from 0.41–0.50 g of ethanol per gram of sugar consumed.

The technology is available to produce fuel ethanol from renewable lignocellulosic biomass. The current challenge is to assemble the various process options into a commercial venture and begin the task of incremental improvement. Current process designs for lignocellulose are far more complex than grain to ethanol processes. This complexity results in part from the complexity of the substrate and the biological limitations of the catalyst. The work of Ingram et al. (1999) at the University of Florida has focused primarily on the genetic engineering of enteric bacteria using genes encoding *Zymomonas mobilis* pyruvate decarboxylase and alcohol dehydrogenase. These two genes have been assembled into a portable ethanol production cassette, the PET operon, and integrated into the chromosome of *E. coli* B for use with hemicellulose-derived syrups. The resulting strain, KO11, produces ethanol efficiently from all hexose and pentose sugars present in the polymers of hemicellulose. By using the same approach, the PET operon has been integrated into the chromosome of *Klebsiella oxytoca* to produce strain P2 for use in the simultaneous saccharification and fermentation (SSF) process for cellulose. Strain P2 has the native ability to ferment cellobiose and cellotriose, eliminating the need for one class of cellulase enzymes. Recently, the ability to produce and secrete high levels of endoglucanase has also been added to strain P2, further reducing the requirement for fungal cellulase. The general approach for the genetic engineering of new biocatalysts using the PET operon has been most successful with enteric bacteria but was also extended to gram-positive bacteria, which have other useful traits for lignocellulose conversion.

Zhang et al. (1995) metabolically engineered the ethanol-producing bacterium *Zymomonas mobilis* to broaden its range of fermentable substrates to include the pentose sugar xylose. Two operons encoding xylose assimilation and pentose phosphate pathway enzymes were constructed and transformed into

Z. mobilis in order to generate a strain that grew on xylose and efficiently fermented it to ethanol. Thus, anaerobic fermentation of a pentose sugar to ethanol was achieved through a combination of the pentose phosphate and Entner–Doudoroff pathways. Furthermore, this strain efficiently fermented both glucose and xylose, which is essential for economical conversion of lignocellulosic biomass to ethanol.

In another study, a technical and economic analysis of the production of fuel ethanol by fermentation of a pentose-rich hydrolyzate with recombinant *E. coli*, strain KO11 was carried out. Hydrolyzate from steam-pretreated willow was used as raw material in calculations regarding the fermentation. The calculations were based on a feed capacity of 10 metric tons of dry willow per hour to the pretreatment stage, providing 35 metric tons of hydrolyzate per hour, consisting of 45 g of sugars L^{-1}, to the pentose fermentation plant. A detoxification step was included, since the hydrolyzate has been shown to have an inhibitory effect on the *E. coli* KO11. The technical data used in the calculations were based on a kinetic fermentation model, which was developed from laboratory-scale experiments in a previous study. The economic analysis predicted an ethanol production cost of 48 cent L^{-1} in the pentose fermentation plant, indicating potentially good economy. The detoxification cost constitutes 22% of this cost. Sensitivity analyses revealed that if the concentration of sugars in the feed to the fermentation was decreased by 40% to 27 g L^{-1}, the ethanol production cost was increased to 54 cent L^{-1}. The production cost was increased to 50 cent L^{-1} ethanol if the cell mass was recirculated to the fermentation stage 5 times instead of 20 (von-Sivers et al., 1994).

Unsorted, mixed waste office paper (MWOP) is an excellent substrate for conversion into fuel ethanol using a recombinant strain of *Klebsiella oxytoca* which ferments cellobiose and cellotriose to ethanol at near theoretical yields, eliminating the need for supplemental β-glucosidase. This organism was tested with commercial fungal cellulase in optimized simultaneous saccharification and fermentation experiments (SSF) using MWOP as a substrate at pH 5–5.2 and at 35 °C. (Brooks and Ingram, 1995).

Many opportunities remain for further improvements in these biocatalysts as we proceed toward the development of single organisms that can be used for the efficient fermentation of both hemicellulosic and cellulosic substrates.

3 Immobilized Cell Systems

Ethanol production from biomass by fermentation is possible by using free or immobilized cells. Both of them have some advantages and disadvantages. Optimum commercial fermentation designs must maintain cell viability while suppressing excess growth, the growth of bacteria and the formation of unwanted products.

The use of immobilized whole cells in industrial processes has attracted considerable attention during the past few years due to advantages over traditional processes (Hamdy et al., 1990). Immobilization is the restriction of cell mobility within a defined space. Immobilized cell cultures have the following advantages over suspension cultures (Shuler and Kargi, 1992):

- Provision of high cell concentrations.
- Elimination of cell washout problems at high dilution rates.
- Elimination of costly processes of cell recovery and cell recycle.
- High volumetric productivities can be obtained due to high cell concentrations and high flow rates (no washout restrictions).
- Provision of favorable microenvironmental conditions such as cell–cell contact, nutrient–product gradients, and pH gradients for cells. In this way better performance of the biocatalysts (e.g., higher product yields and rates) can be obtained.
- Genetic stability may be improved.
- Shear damage is protected and this is important for some cells.

Cell immobilization has also some disadvantages:

- The major limitation on immobilization is that the product of interest should be excreted by the cells.
- Immobilization often leads to systems for which diffusional limitations are important. In such cases the control of microenvironmental conditions is difficult due to the resulting heterogeneity in the systems.
- With living cells, growth and gas evolution present significant problems in some systems; growth and gas evolution can lead to significant mechanical disruption of the immobilizing matrix.

There are mainly two types of immobilization methods: active and passive immobilization of cells. Active immobilization is entrapment or binding of cells by physical or chemical forces. Physical entrapment within porous matrices is the most widely used method of cell immobilization. Various matrices can be used for cell immobilization. Porous polymers (agar, alginate, κ-carrageenan, polyacrylamide, chitosan, gelatin, and collagen), porous metal screens, polyurethane, silicagel, polystyrene, and cellulose triacetate are used. Polymer beads are usually formed in the presence of cells. They should be porous enough to allow the transport of substrates and products in and out of the bead. The beads can be prepared by one of the following methods:

- gelation of polymers,
- precipitation of polymers,
- ion-exchange gelation,
- polycondensation,
- polymerization.

One major problem encountered in immobilizing cells with physical entrapment is the lack of expansibility of the conventional cell carriers; cell growth is restricted in the carrier, resulting in limited catalyst life as well as limited catalyst stability (Joung and Royer, 1990). Conventional carriers such as polyacrylamide gels or carrageenan gels, which have frequently been used for cell immobilization, have limited expansibility.

Encapsulation is another method of cell entrapment. Microcapsules are hollow, spherical particles bound by semipermeable membranes. Cells are entrapped within the hollow capsule volume. The transport of nutrients and products in and out of the capsule takes place through the capsule membrane. Microcapsules have certain advantages over gel beads, such as:

- More cells can be packed per unit volume of support material into capsules.
- Intraparticle diffusion limitations are less severe in capsules due to the presence of liquid cell suspension in the intracapsule space.

Various polymers such as nylon, collodion, polystyrene, acrylate, polylysine-alginate hydrogel, cellulose acetate-ethyl cellulose, and polyester membranes can be used as capsule membranes.

Another form of entrapment is the use of macroscopic membrane-based reactors. The simplest of these types of reactors is the hollow fiber reactor. This device is a mass-transfer analog of the shell and tube heat exchanger in which the tubes are made of semipermeable membranes. Cells are inoculated on the shell side and are allowed to grow in place. The nutrient solution is pumped through the insides of the tubes. Nutrients diffuse through the membrane and are utilized by the cells, and metabolic products diffuse back into the flowing nutrient stream.

Adsorption of cells on inert support surfaces which is another method of active immobilization has been widely used for cell immobilization. The major advantage of immobilization by adsorption is direct contact between nutrient and support materials. High cell loadings can also be obtained using microporous support materials. However, porous support materials may cause intraparticle pore diffusion limitations at high cell densities, as is also the case with polymer-entrapped cell systems. The control of microenvironmental conditions is another problem with porous support materials. A ratio of pore to cell diameter of 4 to 5 is recommended for the immobilization of cells onto the inner surface of porous support particles.

At small pore sizes, accessibility of the nutrient into inner surfaces of pores may be the limiting factor, whereas the specific surface area may be the limiting factor at large pore sizes. So in order to obtain the maximum rate of bioconversion, an optimal pore size should be found. Adsorption capacity and strength of binding are the two major factors that affect the selection of a suitable support material (Shuler and Kargi, 1992). Adsorption is a simple, inex-

pensive method of cell immobilization, but limited cell loadings and rather weak binding forces reduce the attractiveness of this method.

Covalent binding, which is a type of active immobilization method, is the most widely used method for enzyme immobilization. However, it is not widely used for cell immobilization since functional groups on the cell and support material surfaces are not usually suitable for covalent binding (Shuler and Kargi, 1992). Binding surfaces need to be specially treated with coupling agents (such as, glutaraldehyde or carbodiimide) or reactive groups when covalent binding is used. These reactive groups may be toxic to cells and this is an important disadvantage of the binding method. A number of inorganic carriers (metal oxides such as titanium and zirconium oxide) have been developed which provide satisfactory functional groups for covalent binding. Covalent binding forces are stronger than adsorption forces since more stable binding can be obtained. However, with growing cells, large numbers of cell progeny must be lost. The direct cross-linking of cells by glutaraldehyde to form an insoluble aggregate is more like cell entrapment than binding. However, some cells may be cross-linked after adsorption onto support surfaces (Karbaum and Kleine, 1991). Cross-linking by glutaraldehyde may adversely affect the cells' metabolic activity and may also cause severe diffusion limitations. So this method is not widely used.

In passive immobilization, biological films are used. Biological films are a multilayer growth on solid support surfaces. The support material can be inert or biologically active. Biofilm formation is common in natural and industrial fermentation systems, such as in biological wastewater treatment and mold fermentations in which the interaction among cells and the binding forces between the cell and support material may be very complicated (Shuler and Kargi, 1992). In a stagnant biological film, nutrients diffuse into the biofilm and products diffuse out into liquid nutrient medium. Nutrient and product profiles within the biofilm are important factors affecting cellular physiology and metabolism. The thickness of a biofilm is also an important factor affecting the performance of a biotic phase. Thin biofilms will have low rates of conversion due to low biomass concentration, and thick biofilms may experience diffusionally limited growth, which may or may not be beneficial depending on the cellular system and objectives.

There are examples in the literature where yeast cells were immobilized by entrapment in calcium alginate gel beads (Gonzalez and Bravo, 1991), into polymeric hydrophilic matrix (Lorenz et al., 1987), on Berl-saddles by using channeled alumina beads (Hamdy et al., 1990; Garcia-Garcia et al., 1989), or by using delipided and extracted plant vehicles (Karbaum and Kleine, 1991).

Mass transfer limitations resulting from cell immobilization greatly affect the kinetics of ethanol production in these systems. Where entrapment in alginate beads is involved, diffusion of substrate, ethanol, and CO_2 can be enhanced by employing structurally stable beads of small diameter. Small beads

minimize the interfacial areas between solid and liquid phases, and thus permit the maintenance of high concentrations of viable cells. Disruption of bead structure and subsequent cell losses result from the accumulation of CO_2 within beads due to inadequate diffusion.

Grote et al. (1980) have clearly demonstrated the dependence of ethanol productivity and the specific rates of glucose uptake (q_s) and ethanol production (q_p) upon the dilution rate of immobilized bioreactors (Figs. 6 and 7). Dilution rates of approximately 0.8 h^{-1} were found to be optimal for the bacterial fermentations investigated. The high volumetric ethanol productivities characteristic for immobilized systems are shown in Tab. 8. From these data it is evident that in bacterial fermentations the yield of ethanol is higher than in yeast fermentations. The most significant advantage of immobilized cell systems probably is the ability to operate with high productivity at dilution rates exceeding the maximum specific growth rate (μ_{max}) of the microorganism.

Several theories have been proposed to explain the enhanced fermentation capacity of microorganisms as a result of immobilization.

(1) A reduction in the ethanol concentration in the immediate microenvironment of the organism due to the formation of a protective layer or specific

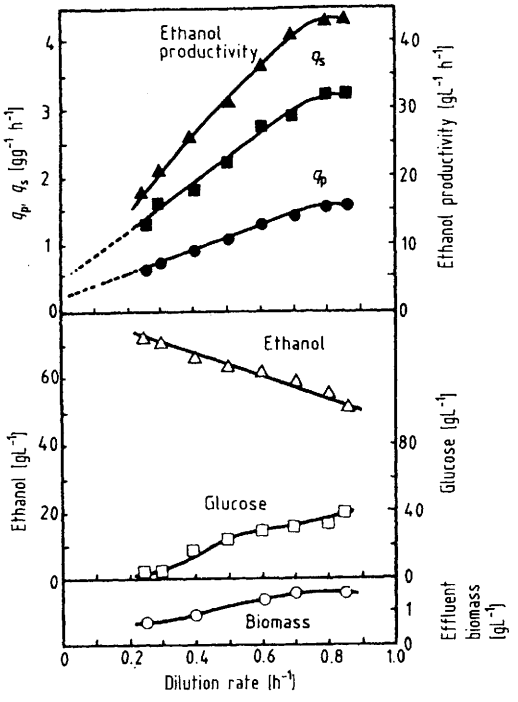

Fig. 6. Effect of dilution rate on fermentation with Ca alginate immobilized cells (Grote et al., 1980).

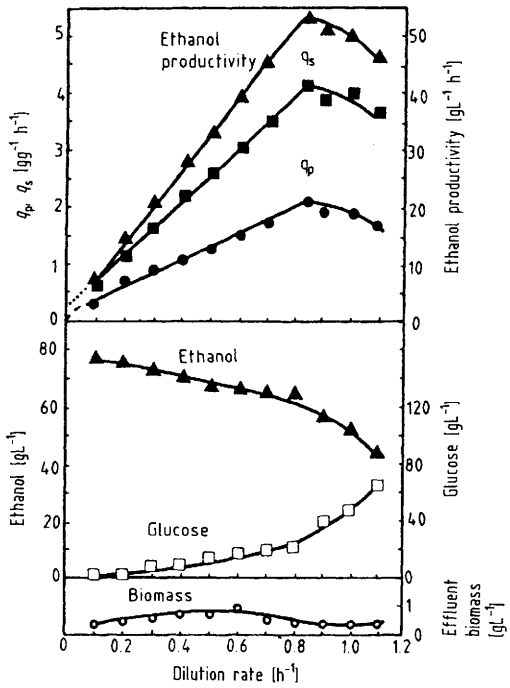

Fig. 7. Effect of dilution rate on fermentation with κ-carrageenan immobilized cells (Grote et al., 1980).

adsorption of ethanol by the support may act to minimize end product inhibition.

(2) Substrate inhibition may be diminished by a gel matrix, if the rate of fermentation meets or exceeds the rate of glucose diffusion to the cell.

(3) Alteration of the cell membrane during the process of immobilization provides improved transfer of substrate into and of product out of the microbe.

The effect of temperature on the rate of ethanol production is markedly different for free and for immobilized systems (Fig. 8). A constant increase in rate is observed with free *Saccharomyces cerevisiae* as temperature is increased from 25–42 °C. With cells immobilized in sodium alginate a maximum occurs at 30 °C. The lower temperature optimum for immobilized systems may result from the diffusional limitations of ethanol within the support matrix. At higher temperatures, ethanol production exceeds its rate of diffusion so that accumulation occurs within the beads. The inhibitory levels then cause the declines observed in ethanol production rate.

Significant differences are also apparent with regard to the effect of pH on the fermentation rate (Fig. 9). The narrow pH optimum characteristic for free cell systems is replaced by an extremely broad range upon immobilization.

Tab. 8. Ethanol Productivity in Immobilized Systems (Saccharomyces cerevisiae and Zymomonas mobilis)

System	Feed Sugar Concentration [g L^{-1}]	Feed Sugar Utilization [%]	Dilution Rate [h^{-1}]	Maximal Ethanol Productivity [g L^{-1} h^{-1}]	Reference
S. cerevisiae carrageenan	glucose 100	86	1.0	43	Wada et al. (1979)
S. cerevisiae Ca-alginate	glucose 127	63	4.6	53.8	Williams and Munnecke (1981)
S. cerevisiae Ca-alginate	molasses 175	83	0.3	21.3	Linko and Linko (1981b)
S. cerevisiae carrier A	molasses 197	74	0.35	25	Ghose and Bandyopadhyay (1980)
Z. mobilis Ca-alginate	glucose 150	75	0.85	44	Grote et al. (1980)
Z. mobilis Ca-alginate	glucose 100	87	2.4	102	Margaritis et al. (1981)
Z. mobilis carrageenan	glucose 150	85	0.8	53	Grote et al. (1980)
Z. mobilis flocculation	glucose 100			120	Accuri et al. (1980)
Z. mobilis borosilicate	glucose 50			85	Arcuri et al. (1980)
Z. mobilis carrageenan-locust bean gum	whey-lactose 50	89		178	Linko and Linko (1981a)

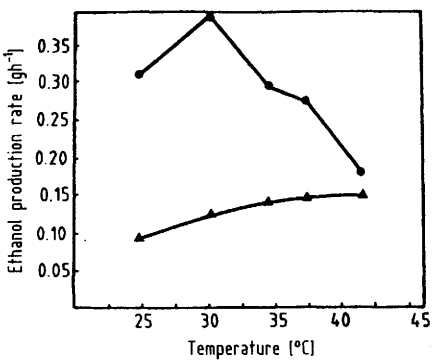

Fig. 8. Relationship between temperature and ethanol production rate for free yeast cell suspensions (-▲—▲-) and immobilized yeast cells (-●—●-) (Williams and Munnecke, 1981).

This effect stems from the pH gradient that exists within the bead (Halwachs et al., 1978). Tab. 9 gives a comparison of the volumetric ethanol productivities of various free and immobilized systems. In terms of ethanol productivity, immobilized cell systems are superior to free cell methods.

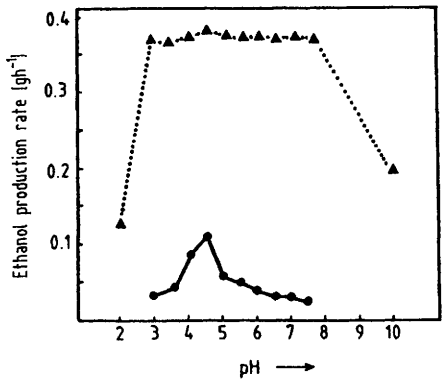

Fig. 9. Relationship between pH and ethanol production rate for free yeast cell suspensions (-●—●-) and immobilized yeast cells (-▲—▲-) (Williams and Munnecke, 1981).

Ethanol production by immobilized yeast cells in a tubular reactor was tried by Qureshi et al. (1987) where the immobilization material was calcium alginate beads and maximum production was obtained in vertical reactors.

Gilson and Thomas (1995) describe the use of yeast, immobilized in alginate beads of known standard size and mechanical strength, in a novel design of fluidized-bed bioreactor. It was found that increasing the alginate concentration in the range of 1–5% (w/w) had little effect on the performance of the immobilized yeast in converting glucose to ethanol, but reduced the tendency of beads to split. Increasing the bead diameter in the range 1–5 mm was found to increase the tendency to split and to reduce the overall conversion of glucose. A model is proposed to describe the consumption of glucose within beads based on Michaelis–Menten kinetics and the diffusion of glucose into beads.

Polyurethanes with hydrophilic properties have been studied by different groups. Lorenz et al. (1987) obtained an efficiency of 82%. Caylak and Vardar-Sukan (1998) used agar, polyurethane foam, and Luffa cylindrica fibers as immobilization materials for *S. cerevisiae*. Calculations for efficiency, yield, and volumetric productivity were carried out and compared with the values obtained using free cells (86%). The efficiencies observed were 78.6%, 96.0%, and 89.4%, respectively for systems using agar, polyurethane foam, and Luffa cylindrica fibers as immobilization materials.

Tab. 9. Ethanol Productivity in Free and Immobilized Systems (with *Saccharomyces cerevisiae*) (Tyagi and Ghose, 1982)

Process	Substrate	Volumetric Ethanol Productivity [g L^{-1} h^{-1}]
Batch	molasses	2.0 (excluding downtime of fermenter)
Continuous (free cell)	molasses	3.35
Continuous (immobilized)	molasses	28.6

4 Substrates for Industrial Alcohol Production

Ethanol can be produced from many different raw materials, which are grouped according to the type of carbohydrates they contain, i.e., sugar, starch, or cellulose. Sugar for ethanol production (sucrose, glucose, or fructose) may be derived from any of the three classes of raw materials (Kohli, 1980). Tab. 1 lists many of the raw materials that have been proposed for fermentation to ethanol (Keim, 1983).

Industrial processes for the production of ethanol by fermentation of molasses, beet, cane, or grain sugars are well established. Since sugars are already available in a degradable form and yeast cells can metabolize sugars directly, these substrates require the least costly preparation. The other carbohydrates must be hydrolyzed to sugars before they can be metabolized. So although starchy or cellulosic materials are cheaper than sugar-containing raw materials, the requirement of converting the starch or cellulosic materials to fermentable sugars is a disadvantage of these substrates. Extensive and costly processes are required to convert starchy or cellulosic substrates to their monomers, sugars. Cheaper raw materials such as spent sulfite liquors, and whey have great potential as sources of fermentable sugars. The sugar concentration in these raw materials is lower than in the agricultural materials and will, in most cases, give dilute sugar solutions. Spent sulfite liquors have a hexose concentration around 20–30 g L^{-1}. Whey has a lactose concentration between 38 and 50 g L^{-1}, depending on whether it is acid or sweet whey, which can be hydrolyzed to the same concentration of glucose and galactose. However, when using cellulosic materials, the sugar concentration depends on the raw material as well as on the process used to convert the cellulose to sugars. Several acid hydrolysis processes give a maximum sugar concentration of around 40–60 g L^{-1} (Zacchi and Axelsson, 1989). The maximum sugar concentration will probably be in the same range for processes based on enzymatic hydrolysis. Starch-containing substrates must also be hydrolyzed before being used for the production of ethanol. In order to convert the starch to sugars, similar to cellulose, enzymatic or acid hydrolysis is used.

On industrial scale, the carbon, hydrogen, and oxygen are normally provided by a complex carbohydrate source such as cane or beet molasses. Additional supplementation with nutrients may be required in some cases. The nutrient such as nitrogen is usually supplied as individual components such as ammonia or ammonium salts, particularly ammonium sulfate. Urea is also used for economic reasons, but it is less readily assimilated unless biotin is also added. Phosphorus is provided as phosphoric acid or ammonium or potassium phosphate, which also provides the potassium. The other media components are normally present with the complex carbon sources, although the magnesium, sulfate, chloride, biotin, and thiamin may be needed for supplementation.

In a study by Qureshi and Manderson (1995) four renewable agricultural resources were considered in a process design analysis for the industrial produc-

tion of ethanol. Raw materials considered were wood, molasses, whey permeate, and starch. Final fermentation substrates were diluted and/or concentrated to give equivalent sugar concentrations for each case. It was found that molasses sugars were cheaper than sugars derived from the other raw materials. However, especially in Europe the amount of molasses is dramatically decreasing due to modern technologies such as the Quentin process (leaving only about 42% sugar), or ion exchange methods (leaving only traces of sugar). The reasons for this are economic as well as ecologic constraints.

4.1 Sugar Crops

4.1.1 Sugarcane

Although sugarcane is grown primarily for sucrose and molasses production, it is also used as a raw material in ethanolic fermentations. It has a high biomass yield of desirable composition as shown in Fig. 10. The crop may be harvested over an extended period of time due to its long growing season.

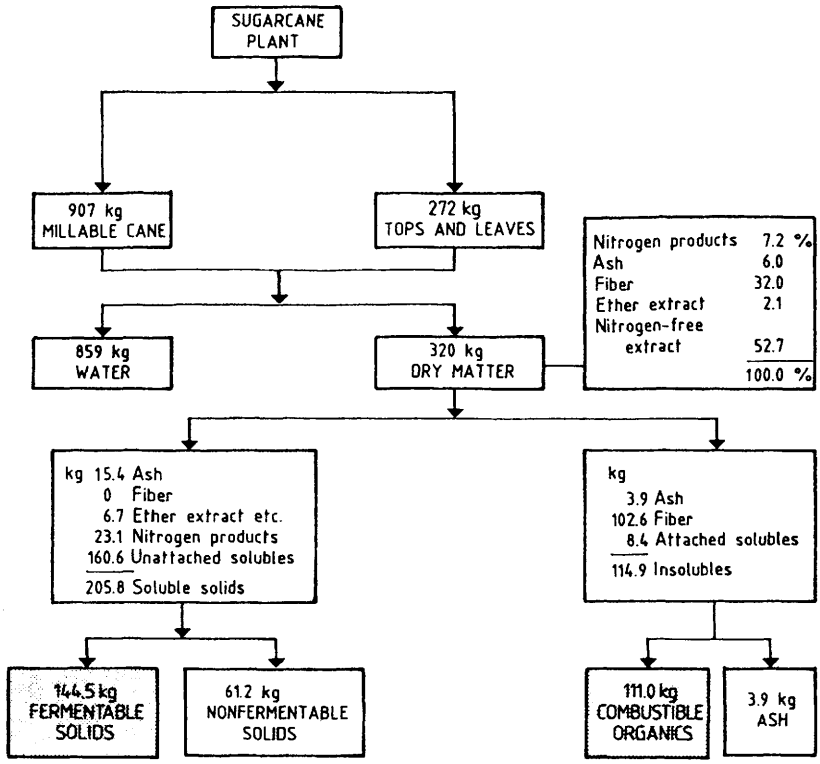

Fig. 10. Typical composition of sugarcane. Fermentable solids include sucrose, starches, and various other carbohydrates; products in shaded boxes are usable for fuel (Nathan, 1978).

The fermentable carbohydrates from sugarcane may be directly utilized in the form of cane juice (central or autonomous distilleries) or in conjunction with a sugar factory from black strap molasses (annexed distilleries) (Kosaric et al., 1980).

Cane juice is prepared by crushing the raw cane and after extraction, clarifying with milk of lime and H_2SO_4 to precipitate the inorganic fraction (Prouty, 1980). The resulting extract is a green, sticky fluid, slightly more viscous than water, with an average sucrose content of 12–13%. It may then be evaporated to the desired concentration and used directly in the fermentation. A major disadvantage in the utilization of sugarcane juice is its lack of stability over extended periods of storage.

Black strap molasses is the non-crystallizable residue remaining after the sucrose has been crystallized from cane juice. This heavy viscous material is composed of sucrose, glucose, and fructose (invert sugars) at a total carbohydrate concentration of 50–60% (w/v). Molasses may be easily stored for long periods of time and diluted to the required concentration prior to use.

4.1.2 Sugar and Fodder Beets

Sugar beet is a more versatile crop than sugarcane since it can tolerate a wide range of soil and climatic conditions. This allows for its successful growth throughout nearly one-half of the United States, Europe, Africa, Australia, and New Zealand. Although the fermentable carbohydrate content is lower than that of sugarcane (Fig. 11), production of this crop per unit area may exceed sugarcane capabilities depending upon the conditions of cultivation.

As for sugarcane, beet molasses is generated in large volumes from the sucrose recovery operation. In Tab. 10 the composition of cane and beet molasses are compared. These raw materials contain sufficient nitrogen and other organic and inorganic nutrients such that little, if any, fortification is required prior to fermentation. Additional benefits from sugar beet come from a high yield of crop co-products such as beet tops and extracted pulp. The pulp is bulky and palatable and has a high feed value in wet or dry form. The tops may be returned to the soil for erosion control and nutrient replacement.

4.1.3 Fruit Crops

Yeast can ferment fruit sugar (usually 6–12% fructose) without pretreatment. Fruits with a high fructose and sucrose content are grapes, peaches, apricots, pears, and pineapples. Though the potential of this raw material is relatively high, its use as a feedstock for fuel alcohol production is not likely due to its significant market value for human consumption. Also, fruits are very perishable and sensitive to damage which may lead to substantial losses of their fermentable sugar content dependent upon the extent of spoilage.

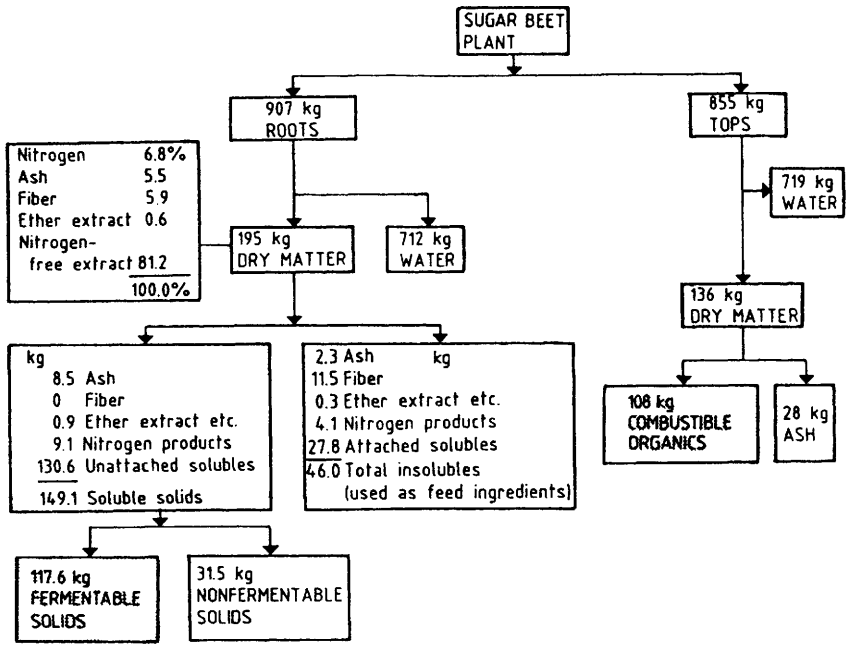

Fig. 11. Typical composition of sugar beets. Fermentable solids include sucrose starches, and various other carbohydrates; products in shaded boxes are usable for fuel (Nathan, 1978).

Tab. 10. Cane and Beet Molasses – Principal Values at 75% Dry Matter (Baker, 1979)

Component		Cane	Beet
Total sugars	[%]	48/56	48/52
Non-sugar organic matter	[%]	9/12	12/17
Sulfated ash	[%]	10/15	10/12
Total organic mattera	[%]	60/65	63/65
Protein, i.e. Np6.25	[%]	2/4	6/10
Sodium	[%]	0.1/0.4	0.3/0.7
Potassium	[%]	1.5/5.0	2.0/7.0
Calcium	[%]	0.4/0.8	0.1/0.5
Chlorine	[%]	0.7/3.0	0.5/1.5
Phosphorus	[%]	0.6/2.0	0.02/0.07
Biotin	[mg kg^{-1}]	1.2/3.2	0.04/0.13
Folic acid	[mg kg^{-1}]	ca. 0.04	ca. 0.2
Inositol	[mg kg^{-1}]	ca. 6,000	5,800/8,000
Ca-pantothenate	[mg kg^{-1}]	54/6.5	50/100
Pyridoxine	[mg kg^{-1}]	2/6.5	ca. 5.4
Riboflavin	[mg kg^{-1}]	ca. 2.5	ca. 0.4
Thiamine	[mg kg^{-1}]	ca.1.8	ca. 1.3
Nicotinic acid	[mg kg^{-1}]	20/800	20/45
Choline	[mg kg^{-1}]	600/800	400/600

[a] Total organic matter is total solids less sulfated ash.

4.2 Industrial and Food Processing Wastes

Industrial and food operation waste streams require some form of treatment to reduce the environmental impact of their disposal. Anaerobic fermentation would not only generate fuel ethanol but also reduce the biological oxygen demand (BOD) of the raw material to an acceptable level. Disadvantages in the utilization of waste materials include the relatively small-scale and widely scattered locations of their production as well as their low carbohydrate concentrations and unfavorable C/N ratios.

4.2.1 Waste Sulfite Liquors (WSL)

Approximately 100×10^6 t a^{-1} WSL are produced as a by-product of the worldwide pulp and paper operations (Forage and Righelato, 1979). This represents ca. 9,180 L WSL per ton of pulp produced. The material arises from the treatment of wood for pulp and from paper production. Although, it should be noted that cellulose industries in Europe have been increasingly switching to closed cycle processes that no more produce any WSL.

As can be seen from Tab. 11, the chief components of WSL are lignosulfonates and hexose as well as pentose sugars. However, there is an inconsistency in the composition of these materials since there is a pronounced difference between softwood (e.g., spruce) and hardwood liquors: the respective portions of hexoses (fermentable) and pentoses is 2:1 in the former and 1:2 in the latter. The BOD_5 value for this material is extremely high (25,000–50,000 ppm). Alcoholic fermentation decreases BOD by 90%, however, the reduction of total organic carbon is low due to the recalcitrant nature of compounds such as lignins, hemilignins, and unhydrolyzed hemicelluloses.

Tab. 11. Chemical Composition of Organic Dry Substance in a Spent Spruce Sulfite Liquor (Detroy and Hesseltine, 1978)

Component	[%]
Lignonsulfonic acids	43
Hemilignin compounds	12
Incompletely hydrolyzed hemicellulose compounds anduronic acids	7
Monosaccharides	
D-Glucose	2.6
D-Xylose	4.6
D-Mannose	11.0
D-Galactose	2.6
L-Arabinose	0.9
Acetic acid	6
Aldonic acids and substances not investigated	10

Pretreatment of this waste before fermentation is minimal. Steam or aeration stripping at pH 1.5–3.0 is required to remove SO_2 which would otherwise inhibit microbial growth. The pH must then be adjusted to optimum and the media supplemented with nitrogen and phosphate nutrients.

4.2.2 Whey

Whey is the liquid effluent generated by the cheese and casein manufacturing industries. The composition is shown in Tab. 12. Sweet whey is a by-product from the manufacture of various hard and soft cheeses whereas acid whey is generated from the production of cottage cheese.

4.2.3 Food Industry Wastes

The characteristics of the food processing industry make large-scale utilization of waste streams improbable. The factors involved in the availability of such residues are as follows:

- highly disperse points of origin;
- seasonal aspects of production (average operation of fruit and vegetable processing plants is about 65% of each year and 75% of total processing is completed in slightly more than 4 months);

Tab. 12. Comparison of Sweet and Acid Whey Composition (Drews, 1975)

Component	Composition in	
	Sweet Whey [%]	Acid Whey [%]
Dry matter	6–7	5–6
Ash	0.5–0.7	0.7–0.8
Crude protein	0.8–1.0	0.8–1.0
Nitrogenous compounds as % of total nitrogen		
genuine protein	52.5	43.9
peptides	31.3	33.1
amino acid	2.5	6.1
creatin	2.6	2.5
ammonia	1.0	2.3
urea	9.1	10.3
purines	1.0	1.8
Lactose	4.5–5.0	3.8–4.2
Lactic acid	traces	up to 0.8
Citric acid	0.1	0.1
pH	4.5–6.7	3.9–4.5

- high variability in both the composition and characteristics of waste streams (solid and liquid);
- predominant need for the addition of nitrogen and added nutrients at added cost;
- generally low sugar concentration (~4%) which is not sufficient for economical ethanol recovery;
- extensive competition with established feed and by-product markets which provide greater cash return for producers of the waste.

4.3 Starches

A variety of starches can be used for ethanol production by fermentation, e.g., grains, cassava (manioc, tapioca), sweet potato, sweet sorghum, and Jerusalem artichoke. Selection of an appropriate substrate depends on a number of factors, not the least of which is the geographical climate of the intended production site. Thus, while corn, wheat, rice, potatoes, and sugar beets are the most common feedstocks in Europe and North America, sugarcane, molasses, cassava, babassu nuts, and sweet potatoes appear to provide the most promising supply of ethanol for tropical countries such as Brazil.

4.3.1 Corn

According to Miranowski (1981), corn is the most viable feedstock for manufacture of ethanol in the United States. The advantages of corn include:

- A relatively high yield.
- A broad geographical cultivation range.
- Corn, like sugarcane, has a C4 photosynthetic mechanism that is inherently quite efficient.
- The energy output-input ratio for corn is higher than for other major crops, with the exception of sugarcane.
- Annual production of corn biomass for all purposes probably exceeds 300×10^6 t (dry basis) in the United States, about 40% of which are residues that presently have little commercial value.

The immediate availability of corn is an important consideration. Extremely efficient systems are already in place for commercially handling corn from seed through culture, harvest, collection, storage, grading, transport, marketing, and shipping, all at very low cost (Kelm, 1980).

As an energy feedstock, three main alternatives exist for the utilization of corn:

(1) The entire corn plant can be harvested for silage and used for energy production.
(2) Utilize corn grain for ethanol production and return the residue to the soil or use it for livestock feed.
(3) Use semi-dried corn plant residues after harvesting of the grain to produce furfural, SNG (substitute natural gas), ammonia, or simple sugars.

In evaluating the potential of corn (and any other food crop) for the production of energy, the moral issue of food vs. fuel must be considered. Approximately 66% of the grain produced in the United States is used as food or feed. The proportion of low-quality ("distressed") grain unsuitable for utilization as food may be as high as 5% of the annual grain production. This material may be suitable for fuel alcohol production.

4.3.2 Cassava

Cassava (*Manihot esculenta*), also called manioc or tapioca, is cultivated in many tropical countries. Brazil, Indonesia, and Zaire are the most important producers. Cassava roots generally contain 20–35 wt.% starch and 1–2 wt.% protein, although strains with up to 38% starch were developed (Chan, 1969). The composition of cassava is shown in Tab. 13.

At a productivity level of 30 t ha^{-1} a^{-1} with 25 wt.% starch and a conversion efficiency of 70%, ethanol yields from cassava are 3,440 L ha^{-1}. Ethanol yields as high as 7,600 L ha^{-1} a^{-1} have been reported (Anonymous, 1980b).

The advantages of cassava as an energy crop for fuel alcohol production include:

- Potential for high alcohol yields per ha of land.
- Lower soil quality required than for sugarcane, therefore, vast areas of little used land available for cultivation.
- High tolerance to draught and disease.

Tab. 13. Composition of Bitter Cultivars of Cassava (de Menezes et al., 1978)

Component	g per 100 g Dry Matter
Starch	80–89
Total sugars	3.6–6.2
Reducing sugar	0.1–2.8
Pentosans	0.1–1.1
Fiber	1.7–3.8
Protein	2.1–6.2
Fat	0.2–0.7
Ash	0.9–2.4

- Available for 48 h before serious deterioration between crop lifting and processing.
- Cassava chips can be easily dried by boiler gases to a moisture content $<20\%$ for stable storage of up to one year.
- Using amylolytic enzymes, good conversion yields are attainable at reasonable cost.
- In mash fermentations, no acids or nutrients are required so that the process can be operated at pH values near neutral; corrosion is thus minimized.
- The high crude protein content of cassava leaves and stems (17%) makes production of a valuable animal feed by-product feasible.

4.3.3 Sweet Potato

With a higher starch yield per unit land cultivated than corn, sweet potato (*Ipomoea batatas*) represents a fuel crop of significant potential. Sweet potato powder (SPP), generated by freeze drying, then grinding and screening the tubers, has a starch content of 64.4% on a dry weight basis (Azhar, 1981).

4.3.4 Sweet Sorghum

A member of the grass family, sweet sorghum (especially *Sorghum saccharatum*) is a valuable energy crop containing both starches and sugars. Its composition is given in Fig. 12.

With currently available cultivars, ethanol yields of 3,500–4,000 L ha^{-1} can be obtained from the fermentable sugars alone. An additional 1,600–1,900 L can be produced from stalk fibers. More than 17,000 lines of sorghum are known to exist. With hybrid strains, it is anticipated that yields may be increased 30% above present levels (McClure et al., 1980).

Among the attributes of this plant that make it a viable candidate for energy production are:

- its adaptability to the majority of the world's agricultural regions,
- its resistance to draught,
- its efficient utilization of nutrients.

4.3.5 Jerusalem Artichoke

The Jerusalem artichoke (*Helianthus tuberosus*, in Europe often called "Topinambur") is related to the sunflower and contains a widespread root system that produces tubers. The plant is native to North America and was introduced

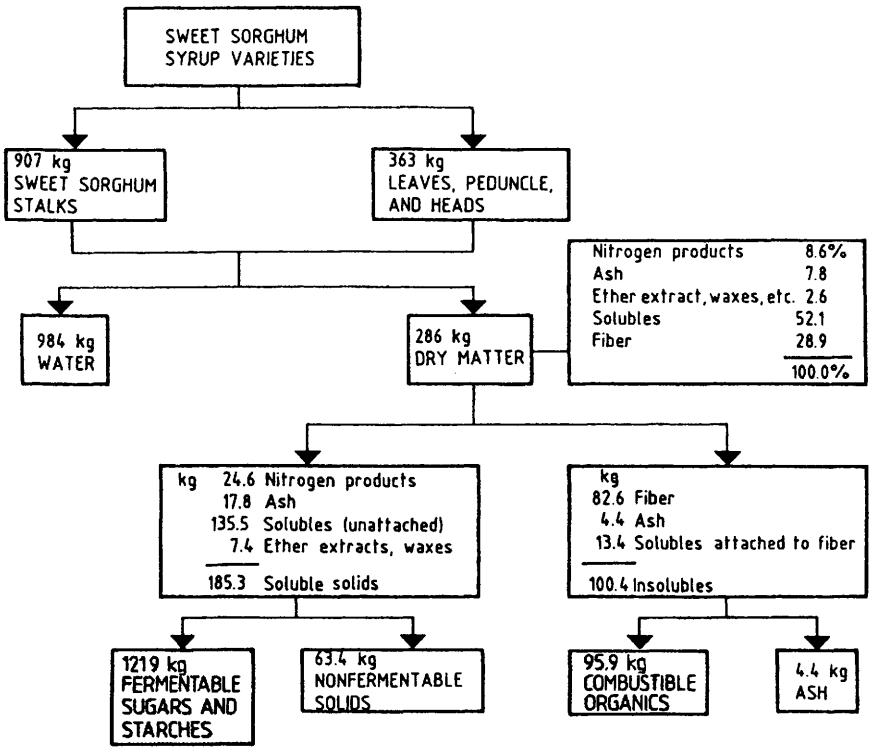

Fig. 12. Estimated composition of sweet sorghum, syrup varieties (Nathan, 1978). Average yields per ha: stalks 47.4 t; leaves, peduncles, and heads 19.3 t; total 66.7 t.

to Europe in the 17th century. It grows better in poor soils than most crops. The plant is resistant to pests and common plant diseases as well as to cold temperatures, which makes it a hardy perennial for cold climates.

The interest in this crop stems mainly from its high productivity and relatively high carbohydrate content. The average yield under optimum conditions is 9 t ha^{-1} (d.m.) of forage and 45 t ha^{-1} (80% moisture) of tubers with approximately 18% (wet basis) total reducing sugar after inulin hydrolysis (Chubey, B. B., Agriculture Canada, Morden, Manitoba, personal communication, 1981).

The carbohydrates in Jerusalem artichokes are mainly fructofuranose units in the form of inulin which consists of linear chains of approximately 35 D-fructose molecules united by β-(2,1) linkages and terminated by a D-glucose molecule which is linked to fructose by an α-(1,2) bond as in sucrose (Bacon and Edelman, 1951; Eihe, 1976). Breakdown products of inulin and other similar carbohydrates are also present but studies have shown that the fructose–glucose ratios seldom fall below 80% : 20% (Kierstan, 1980).

Because of the high carbohydrate content in the tubers, Jerusalem artichoke can serve as a good source of fermentable sugars for production of ethanol. At

80–90% conversion efficiency, it has been suggested that ethanol yields of 3,900–4,500 L ha^{-1} are attainable (Hayes, 1981). The amount of alcohol obtainable per ha of sugar beet, corn and wheat, and Jerusalem artichoke would yield 1.7, 2.0, and 3.7 times more alcohol, respectively (Stauffer et al., 1975). Pulp obtained after extraction of juice for alcohol fermentation can serve as a nutritional animal feed.

4.3.6 Starch Saccharification

4.3.6.1 Enzymatic Hydrolysis of Starch

Two main groups of enzymes are involved in the enzymatic degradation of starch. The first consists of enzymes which split the α-(1,4) bonds between glucose residues. This group can be further classified into:

- endo-enzymes which produce random or internal breaks, and
- exo-enzymes which act from chain ends.

The exo-enzymes catalyze the specific hydrolysis of the α-(1,6) interchain linkages of amylopectin. The details of the specific enzymes and their modes of action are given in the chapter by Senn and Pieper in this book.

4.3.6.2 Acid Hydrolysis of Starch

In acid hydrolysis, the breakdown of starch to glucose is accompanied by further degradation of the sugar to 5-hydroxymethyl furfural, levulinic acid, and formic acid (Kerr, 1944). Acid concentration and type, temperature, and starch concentration have been shown to be key factors in the relative yields of glucose by-products (Azhar, 1981). Although acid hydrolysis of cassava starch has been shown (De Menezes, 1978) to provide more than 98.8% of reducing sugars, the procedure is not recommended because of low alcohol yields (approximately 75% of the theoretical value) due to the presence of non-fermentable and/or inhibitory by-products.

4.4 Lignocellulose

The potential of utilizing global reserves of lignocellulosic materials for conversion to useful fermentation products such as fuel ethanol has generated extensive interest in the past decades. By far, world production and present stocks of cellulose-based biomass are larger in volume than any other carbohy-

drate source. Given an average annual value for incident solar energy reaching the earth's surface estimated to be 3.67×10^{21} kJ a^{-1} (Holdren and Ehrlich, 1974), global photosynthesis (with an efficiency of 0.07%) could convert 2.57×10^{18} kJ a^{-1} to cellulose containing biomass. This efficiency would yield an annual net production of 1.8×10^{11} t of biodegradable material, 40% of which is cellulose (Whittaker, 1970). A production of $1\text{–}1.25 \times 10^{11}$ t a^{-1} of terrestrial dry mass together with $0.44\text{–}0.55 \times 10^{11}$ t a^{-1} in the oceans is estimated (Slesser and Lewis, 1979).

The U.S. Department of Energy (DOE) is promoting the development of ethanol from lignocellulosic feedstocks as an alternative to conventional petroleum transportation fuels. Programs being sponsored by DOE range from basic research to develop better cellulose hydrolysis enzymes and ethanol-fermenting organisms, to engineering studies of potential processes, to co-funding initial ethanol from lignocellulosic biomass demonstration and production facilities. Wright (1988) and Wyman (1996) provide good reviews on the topic of ethanol production from lignocellulosic biomass.

Lignocellulose (wood, grasses, and municipal solid waste) is an attractive feedstock for ethanol production because of its availability at low cost and large quantities. Although biological processes are inherently efficient, the price of this efficiency is the need to process each major component of lignocellulose separately. For example, the cellulose is difficult to hydrolyze to glucose, but it is simple to ferment the glucose to ethanol. Similarly, the hemicellulose (primarily xylose in hardwoods and grasses) is easily broken down to monomeric sugars at high yields, but the xylose is difficult to ferment to ethanol. Lignin is a phenolic polymer and once separated from the lignocellulosic matrix must be processed catalytically to yield useful products.

In the overall process for producing ethanol from wood, the feedstock is pretreated and the xylan and possibly lignin are removed for separate fermentation or chemical processing. The cellulose is hydrolyzed to glucose either by acid or enzyme, and the glucose is then fermented to ethanol.

4.4.1 Characteristics of Lignocellulosic Material

Cellulose is a linear homopolymer of anhydroglucose units linked by β-(1,4) glucosidic bonds. The length of the macromolecule varies greatly as to the source and degree of processing it has undergone. Newsprint, e.g., exhibits an average DP (degree of polymerization) of about 1,000 while cotton is found to have a DP of approximately 10,000 (Callihan and Clemmer, 1979).

It is not this primary structure which makes cellulose such a hydrolysis-resistant molecule. It rather seems to be the effect of secondary and tertiary configurations of the cellulose chain as well as its close association with other pro-

tective polymeric structures within the plant cell wall such as lignin, starch, pectin, hemicelluloses, proteins, and mineral elements.

The hemicellulose and lignin components of the woody fiber are located between the microfibrils or inter-laminar spaces. Hemicelluloses are heteropolymers of galactose, mannose, xylose, arabinose, and various other sugars as well as their uronic acids. Next to cellulose, they are the most abundant organic material on earth. For the economic viability of any process involving the utilization of lignocellulosics, a means of hemicellulose recovery and/or assimilation must be included.

The lignin component of cellulosic-based biomass is responsible to a great extent for the difficulties inherent in cellulose hydrolysis. This macromolecule of phenolic character is the dehydration product of three monomeric alcohols, *trans-p*-coumaryl alcohol, *trans*-coniferyl alcohol, and *trans*-sinapyl alcohol. The relative amounts of each vary with the source. The lignin matrix forms a protective sheath around the cellulose microfibrils. While lignin accounts for some degree of protection to the microfibrils, the conformation of native cellulose also influences hydrolysis.

When cotton cellulose (in its native form) is treated with dilute acid, partial hydrolysis occurs rapidly with about 15% of the cellulose chain being degraded to glucose. The remaining 85%, which is resistant to hydrolysis, is found to exist as rod-shaped particles about 400 Å long and 100 Å wide (Callihan and Clemmer, 1979). These particles correspond to the leveling off degree of polymerization (LoDP) for cellulose and are postulated to exist as regions of highly ordered crystallinity whereas the easily degraded regions are amorphous in nature and quickly hydrolyzed.

Two models have been introduced to explain the above and other observed experimental evidence (Fig. 13). The fringed fibrillar model (Scallan, 1971)

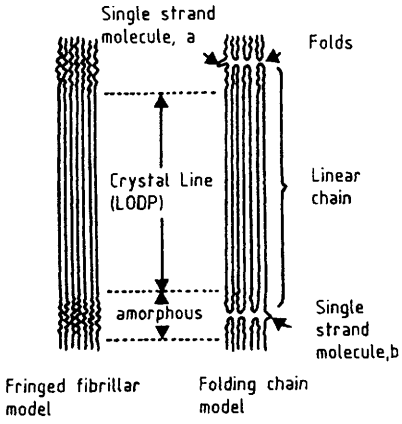

Fig. 13. Fringed fibrillar model and folding chain model of cellulose (Chang et al., 1981).

postulates that cellulose molecules in the elementary fibril are fully extended with the molecular direction completely in line with the fibril axis. Crystalline regions are 500 Å in length and are intermittent with each amorphous region. The folding chain model as developed by Chang (1971) is visualized as the cellulose molecule folding back and forth along the fibrillar axis with a fold length of LoDP. One repeating unit (termed platellite) corresponds to about 1,000 DP and is designated as the chain length between points a and b in Fig. 13. Platellite units are joined in sequence by single-stranded glucose chains which are easily hydrolyzed. A conventional crystallite is comprised of several platellites packed in registry with amorphous regions at the ends and crystalline regions towards the center. The amorphous regions are said to be comprised of the areas rich in deflected β-glucosidic linkages found at each fold in the cellulose chain with 3 monomers per 180° turn. These areas would be more susceptible to hydrolytic attack than the linear crystalline areas due to the thermodynamic properties of the deflected covalent bonds and lack of H-bonding.

Elementary fibrils formed by the close packing of the crystallite units are further associated into larger groups known as microfibrils. The microfibril is about 25 nm across and infinitely long. It is these tightly packed structures that are further surrounded by the lignin matrix.

4.4.2 Pretreatment

A number of physical and/or chemical methods can be used to separate cellulose from its protective sheath of lignin and increase the surface area of the cellulose crystallite by size reduction and swelling. According to new research from a Swedish chemical engineering team, producing ethanol from renewable sources appears to be economically feasible. The team studied ethanol production from willow wood, which was broken down into fibers by steam. Following hydrolysis of the cellulose fibers, the resulting sugars were fermented to produce ethanol for distilling (Anonymous, 1994).

4.4.2.1 Milling

It has been found that while hammer milling produces desirable size reduction and increases the bulk density of the cellulosic material, it does not increase the susceptibility of the cellulose to hydrolysis (Mandels et al., 1974). Compression (two roll) milling is a rapid method (5 min and less) to disrupt crystallinity, lower the DP of the cellulose, and increase the bulk density of the material. Increase in bulk density allows cellulose slurry concentrations of 20–30% to be prepared without detrimental agitation or mass transfer effects (Spano et al., 1979).

Ball milling operations have also been utilized to overcome the lignin barrier. Cellulosic materials have been reduced to sizes of 400 mesh and less by this method (Wilke and Mitra, 1975) and no other pretreatment was found necessary for efficient attack by cellulases (Cowling and Kirk, 1976).

Gentle wet milling has been employed with simultaneous enzymatic degradation of cellulose feedstocks (Kelsey and Shafizadeh, 1980). This co-current attrition and hydrolysis has been found by Neilson et al. (1982) to increase the extent of glucose liberation by 80% over that of conventionally ball milled CF-11 cellulose.

4.4.2.2 Steam Explosion

Steam explosion involves the steam heating of green wood chips to approximately 180–200 °C for 5–30 min in a continuous operation, or at higher temperatures (245 °C) for a shorter time (0.5–2 min) in the batch mode. These methods are known as the Stake and Lotech processes, respectively (Wayman, 1980). During steam treatment, acids are formed by the decomposition of hemicellulose under high temperature and pressure which then serve to catalyze the depolymerization of intact hemicelluloses and lignin (termed "autohydrolysis"). Upon completion of the heating cycle, at which point the lignin is sufficiently softened, the reaction vessel is abruptly discharged to atmospheric pressure, which causes an explosion of the woody cells. This removes lignin from its close association with cellulose and increases the surface area available for catalytic hydrolysis. Residual lignin and hemicelluloses are then easily extracted from the treated product.

Tab. 14 illustrates the enhanced enzymatic hydrolysis parameters for steam exploded hardwoods and crop residues. Increased rates of hydrolysis were not observed for softwoods or municipal wastes. Further advantages of steam explosion include the limitation of undesirable by-product formation and the lack of a need for corrosion proof equipment (Buchholz et al., 1981).

Stake Technology has developed "steam explosion" technology on a commercial basis for recycling wood, crop residues, wastepaper, and municipal solid waste (MSW). It was found that three conditions were necessary for optimal steam explosion: high temperature, a continuous process, and an explosive discharge to quench the reaction. Thus, the StakeTech system has a co-ax feeder, a continuous digester, and a discharge mechanism. The combination of a 10-inch co-ax feeder and a 36-inch digester provides a capacity of 12 metric tons h^{-1} for wood chips at 50% (w/w) moisture content. The technology is currently being used to recycle wastepaper, to convert wastepaper into fuel ethanol, to convert the organic fraction of MSW into a soil enhancer, and to produce cattle feed from agricultural wastes and waste hardwoods (Taylor et al., 1995).

Tab. 14. Effect of Steam Pretreatment on the Enzymatic Hydrolysis[a] of Various Cellulosic Substrates (Spano et al., 1979)

Substrate	Pretreatment	Total Reducing Sugars [mg mL^{-1}]	
		4 h	24 h
Hardwoods			
Poplar	None	1.4	2.4
	Steam	15.3	25.8
Aspen	None	1.8	3.0
	Steam	12.8	24.8
Agriculture residues			
Corn stover	None	4.9	7.8
	Steam	15.7	22.5
Sugarcane bagasse	None	1.7	2.5
	Steam	9.5	16.1
Urban waste	None	10.5	18.0
	Steam	6.2	10.8
Softwoods			
Eastern spruce	None	2.0	3.8
	Steam	3.5	6.4
Douglas fir	None	1.6	3.2
	Steam	2.8	4.3

[a] *Trichoderma reesei* cellulase (QM9414), 19 IU per g substrate, 5% substrate slurries, pH 4.8, 50 °C, steamed substrates washed prior to enzymatic hydrolysis.

4.4.2.3 Use of Solvents

With the use of appropriate solvents it is possible to selectively remove either lignin or cellulose from the native matrix. This not only serves to dissociate cellulose from its protective lignin covering but also destroys the crystalline structure of native cellulose by successive dissolution and regeneration to a highly active form.

The cost of solvent pretreatment steps has been estimated as the second most important expense in a cellulose saccharification process. Thus, if solvent can be recovered and recycled, this would improve the economic characteristics of the entire operation.

With this recycling aspect in mind, studies have been performed utilizing the solvent Cadoxen, an aqueous alkaline solution of ethylene diamine, and cadmium oxide (Ladisch et al., 1978; Tsao, 1978). After cellulose solubilization, the addition of excess water to the solution causes the reprecipitation of the cellulose as a soft floc, which is then easily removed and hydrolyzed. The Cadoxen solvent may be recycled. Other solvents include HCl, H_2SO_4, H_3PO_4, and alkali. Recently it was reported that Cadoxen was replaced with sulfuric acid.

4.4.2.4 Swelling Agents

Pretreatment agents have been studied for their ability to swell the cellulose matrix and thus open the interior of the fibril to easy attack by the enzymes.

Two types of swelling may take place, intercrystalline and intracrystalline. Intercrystalline swelling is induced by the presence of water and is necessary for any microbial activity to occur. Intracrystalline swelling requires a chemical reagent capable of breaking down the H-bonding of adjacent glucose molecules in the cellulose matrix. These swelling agents include concentrated NaOH, organic bases (i.e., amines), and certain metal salts such as $SnCl_4$ (Chang et al., 1981). Many of the solvents previously discussed also exhibit swelling properties, however, in general, swelling agents improve hydrolysis to a much lesser extent than solvents (Millett et al., 1954) and as well require higher quantities of chemical reagents. The application of heat in conjunction with swelling pretreatment may enhance later hydrolysis to a much greater extent than pretreatment at room temperature (Mandels et al., 1974).

The greatest drawback of these chemical pretreatments (as opposed to physical methods) is that the treated product is at a very low bulk density and, as such, suspensions of 4–5% are too thick to agitate or transport. The processing of cellulose concentrations at this level is not economical for ethanol recovery.

4.4.2.5 Lignin-Consuming Microorganisms

There are a number of microorganisms which produce enzymes required for lignin degradation. Many of these belong to the group of white-rot fungi. Among the wood-rotting fungi – about 2,000 species – more than 90% are white-rot fungi. Tab. 15 lists some of the better known organisms that are able to decompose lignin. White-rot fungi have been studied increasingly in view of their useful actions in the pulp and paper industry, e.g., in biological pulping and bleaching, deinking, or in effluent treatments. The best studied organism is *Phanerochaete chrysosporium*.

Tab. 15. Examples of Microorganisms Capable of Degrading Lignin

Fungi	Bacteria
Phanerochaete chrysosporium	*Nocardia* sp.
Polyporus brumalis	*Streptomyces* sp.
Polyporus versicolor	*Pseudomonas* sp.
Trametes sp.	*Flavobacterium* sp.
Poria sp.	

The use of these organisms may provide important by-product credits in ethanol production (e.g., single cell protein, SCP) as well as remove the protective lignin coat. Much less harsh conditions are required for this type of biological pretreatment than for other chemical methods. As such, side reactions are reduced and production of inhibitory agents is limited.

4.4.3 Acid Hydrolysis

Commercial operations which have produced ethanol from cellulosic material have classically utilized acid hydrolysis for the release of fermentable sugars from the native feedstock.

Acid hydrolysis may be categorized under two general approaches, that of high acid concentration at a low temperature or that of low concentration at a high temperature. The relative advantages/disadvantages of each stem from a trade-off between an increased rate and overall yield of hydrolysis vs. the degradation of glucose to undesirable by-products.

Excellent accounts of the main developments during the first half of the 20th century may be found in Prescott and Dunn (1959), chapters 4 and 46, and Riehm (1962) as well as Conrad (1962).

Historically, the first commercial processes were developed by Classen (1901), employing sulfurous acid, and by Ewen and Tomlinson (1909), working with dilute sulfuric acid. Respective plants were in operation until the end of World War I. Yields of these processes were low, in the range of 75–130 L t^{-1} wood d.m.. In the early 1920s, Scholler (1923) investigated the theoretical and subsequently the technical details of wood hydrolysis with dilute sulfuric acid. The so-called Scholler-Tornesch process, yielding about 200 L t^{-1}, was developed to large-scale operation in the 1930s with plants at Tornesch (13 kt wood d.m. a^{-1}), Dessau (42 kt), Holzminden (24 kt), and Ems, Switzerland (35 kt) as well as in Korea. Whereas these were closed down at the end of the 1950s, in the USSR, about 14 plants with a total capacity of 700 kt a^{-1} were in operation much longer, had processed 4 million tons wood d.m. until 1965, and it is not known when they were closed. During World War II, plants employing a modified Scholler process were erected in the USA at Madison and at Springfield, but were closed down by about 1950/51. In 1950, however, Tennessee Valley Authority (TVA) erected a new plant at Wilson which was mainly producing fodder sugar. It was closed down soon after, caused by heavy price reductions of molasses.

Processes employing concentrated acids have a similar history. The solubility of cellulose in concentrated sulfuric acid had been detected already in 1815. The first industrial process, however, was developed in 1942/43 and run in Italy (Bolzano, Bozen), but had to be closed at the end of World War II. Thirty years later, the technology was rediscovered and reexamined by the Hokkaido

Forest Research Institute in Japan. After carefully studying the details of the process itself, main efforts were successfully directed towards techniques of complete recovery of the sulfuric acid.

The application of concentrated hydrochloric acid for the hydrolysis of (lig-no)cellulosic materials defines another process variant which was developed in the 1920s and 1930s by Hägglund, Bergius, Koch, and Specht in Germany, known as the Bergius–Rheinau process. Details of this process and variants which was mainly applied to produce sugar and was in operation until the 1960s, may be found, e.g., in the articles of Riehm (1962) and Conrad (1962).

4.4.3.1 Concentrated Acid

Crystalline cellulose is completely soluble in 72% H_2SO_4 or 42% HCl solutions at relatively low temperatures (10–45 °C) (Oshima, 1965). The polymer is depolymerized to yield oligosaccharides, the bulk of which is cellulotetraose. Little, if any, glucose monomers are released at this stage. After dissolution in the concentrated acid, the oligomer mixture is then diluted to a lower concentration and heated to about 100–200 °C for 1–3 h. This step converts oligomeric glucose chains to their monomeric constituents.

Acid hydrolysis kinetics at high acid concentrations do not depend upon the structural details or crystallinity of the cellulose substrate. As such, yields >90% of potential glucose may be obtained (Grethlein, 1978).

The main drawback in the use of concentrated acids is that the acid must be recovered and recycled to be economically effective. Such recovery operations are generally cost intensive. The use of HCl has a technical advantage over H_2SO_4 in this regard since it is a volatile acid and may be recovered by vacuum stripping methods. Though vacuum recovery of H_2SO_4 is not feasible, its use may be integrated with another system with profitable results. An example of this would be the use of the neutralization product $CaSO_4$ in the manufacture of gypsum. Additional expenses arise in concentrated acid processes due to the requirement for corrosive-resistant vessels and large reactor volumes per unit of production due to long reaction times.

4.4.3.2 Dilute Acid

While concentrated acid hydrolyzes cellulose rapidly with little or no requirement for pretreatment, the yield of fermentable sugars is usually quite low due to degradation of the glucose as it is released from the polymer. Dilute acid processes yield less degradation products, however, the rate of hydrolysis is lower due to the effect of the resistant crystalline regions within cellulose.

Scholler (1923), in Germany, and Saeman (1945) of the U.S. Forest Products Laboratory predicted the time course of cellulose hydrolysis catalyzed by 0.4–1.6% H_2SO_4 (aq.). The kinetics were found to be 1st order and involved 2 reaction steps:

$$\text{Cellulose} \xrightarrow{K_1} \text{Sugar} \tag{1}$$

$$\text{Sugar} \xrightarrow{K_2} \text{Decomposition products} \tag{2}$$

The rate constants (K_1, K_2) are related to temperature by traditional Arrhenius reaction kinetics. Thus, the fraction of net reducing sugars liberated over that of the potential from the cellulose feedstock may be described as a function of time for any temperature profile and acid concentration by Eq. (3).

$$\frac{C_B}{a} = \frac{K_1}{K_2 - K_1}\left(e^{K_1 t} - e^{K_2 t}\right) \tag{3}$$

where C_B is the net reducing sugar formed, a is the initial cellulose concentration (as glucose equivalents per 100 g starting material), t time, and K_1, K_2 reaction rate constants.

The activation energies for the decomposition of cellulose and glucose are 189,000 and 137,000 kJ mol^{-1}, respectively (Ghose and Ghosh, 1978) and thus, the values of K_1 and K_2 are nearly equivalent. Although $C_B\, a^{-1}$ is found to increase with increasing temperature and acid concentration, substantial degradation products are formed under these conditions. Fig. 14 shows acid hydrolysis curves at 180 °C using a 0.6% H_2SO_4 solution. The ordinate indicates the release of reducing sugars as a percentage of the potential glucose in the cellulose chain. If no degradation products are formed, the reaction would proceed

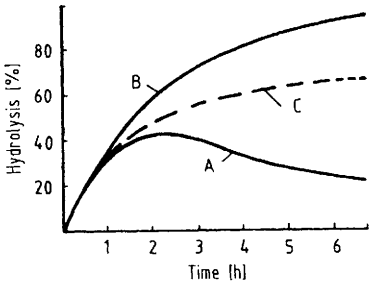

Fig. 14. Formation of glucose by the dilute acid hydrolysis of cellulose (Wettstein and DeVos, 1980).
A: hydrolysis kinetics according to Saeman (1945);
B: ideal hydrolysis kinetics (no degradation);
C: real hydrolysis kinetics.

according to curve B (ideal kinetics). Curve A shows the time course for material obeying Saeman kinetics (see Eq. (3)). The real behavior of wood saccharification processes lies in the area between these two limits as illustrated by the broken line. Technical processes which utilize dilute H_2SO_4 yield approximately 65–80% of the total reducing sugar.

Kinetic data have shown that the rate of sugar release using dilute acid can be substantially improved by operating at very high temperatures ($\sim 500\,^{\circ}C$) and for short times (Yu and Miller, 1980). The detrimental effects of hemicellulose side reactions which form compounds inhibitory to the subsequent fermentation is a major drawback in these high-temperature processes.

To approach the ideal hydrolysis curve B in Fig. 14, sugar should be removed as soon as it is liberated from the cellulosic chain. To achieve this, an infinite amount of extraction liquid must be provided to the hydrolysis medium. This would amount to a sugar concentration of near zero in the processed stream. Wayman (1980) was able to minimize this effect by carrying out a number of hydrolysis stages on steam exploded aspen using 2% H_2SO_4 at 190 °C for a period of 20 min at each stage. Each limited hydrolysis step liberated about 20% of monomers from the cellulose chain. If free sugar residues are removed at the termination of each partial effect, the overall yield of reducing sugars can reach values in excess of 90% after 5 cycles (Fig. 15).

Overall costs for processes which utilize dilute acids are much less than that for concentrated acids. Therefore, acid recycle is not necessary for the economic viability of the process.

Generally, disadvantages with dilute acid include low sugar yields, high energy consumption due to hydrolysis at elevated temperatures and pressures, and (though less so than concentrated acids) the need for corrosion-resistant materials.

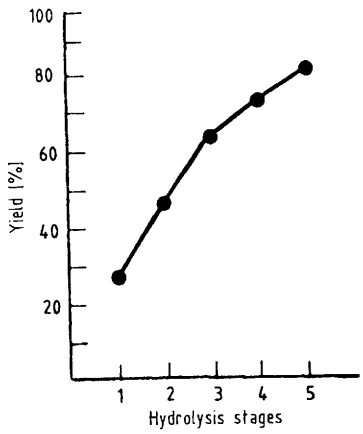

Fig. 15. Multistage hydrolysis (yield in % of theoretical) of autohydrolyzed, extracted aspen, employing 2% H_2SO_4 on fiber at 190 °C for 20 min for each stage (Wayman, 1980).

The effect of combined heat treatment and acid hydrolysis (various concentrations) on cassava grate waste (CGW) biomass for ethanol production was investigated (Agu et al., 1997). At high concentrations of H_2SO_4 (1–5 M), hydrolysis of the CGW biomass was achieved but with excessive charring or dehydration reaction. At lower acid concentrations, hydrolysis of CGW biomass was also achieved with 0.3–0.5 M H_2SO_4, while partial hydrolysis was obtained below 0.3 M H_2SO_4 (the lowest acid concentration that hydrolyzed CGW biomass) at 120 °C and 1 atm pressure for 30 min. A 60% process efficiency was achieved with 0.3 M H_2SO_4 in hydrolyzing the cellulose and lignin materials present in the CGW biomass. It was concluded that high acid concentration is not required for CGW biomass hydrolysis. The low acid concentration required for CGW biomass hydrolysis, as well as the minimal cost required for detoxification of CGW biomass because of low hydrogen cyanide content of CGW biomass seemed to make this process very economical. From 3 L of the CGW biomass hydrolyzate obtained from hydrolysis with 0.3 M H_2SO_4, ethanol yield was 3.5% (v/v) after yeast fermentation. However, although the process resulted in gainful utilization of CGW biomass, additional costs would be required to effectively dispose new by-products generated from CGW biomass processing.

4.4.4 Enzymatic Hydrolysis

Cellulases, microbial enzymes capable of cellulose hydrolysis, are in reality a number of several different synergistic components. The cellulase complex is found to consist of 3 basic components which may be present in multiple forms, often as isoenzymes (Bisaria and Ghose, 1981). Traditionally, these groups of enzymes have been classified as:

(1) *endo*-β-(1,4)-glucanases (CMC^{ase}) consist of several components varying in their degree of randomness, they are postulated to cleave β-(1,4) glucosidic linkages in native cellulose;
(2) *exo*-β-glucanases ($Avicel^{ase}$) consist of specifically two groups:
(3) β-(1,4) glucanase glucohydrolase which removes glucose units from the non-reducing end of the cellulose chain,
(4) β-(1,4) glucan cellobiohydrolase which removes cellobiose units from the non-reducing end of the chain;
(5) β-(1,4) glucosidase hydrolyzes cellobiose and short-chain oligosaccharides to glucose.

In the recent years, parallel to the studies of the main cellulase producer, *Trichoderma reesei* there has been some change of designations. Since *T. reesei* enzymes are known to be typical for fungal cellulases, the new grouping is:

(1) endoglucanases hydrolyzing internal bonds in disordered regions along fibers,

(2) cellobiohydrolases attacking ends of products of (1) with release of cellobiose,
(3) β-glucosidase yielding glucose

T. reesei is reported to contain two enzymes each of the first two categories, EG I and II, and CBH I and II with the respective genes, and there is less data regarding the number of β-glucosidases.

The purification and characterization of these proteins has been carried out with a number of organisms from different sources. It is difficult to generalize on the physical properties of the enzymes since they are subject to a high degree of variation. Their molecular weights commonly range from 12,000–80,000 with β-glucosidase generally being the larger macromolecule.

Cellulases are induced enzymes and are produced only when the organism is grown in the presence of cellulose, cellobiose, lactose, sophorose, or other glucans which contain β-(1,4) linkages (Gratzali and Brown, 1979). These enzymes are also highly regulated by end-product inhibition. This phenomenon has led to interest in the continuous removal of hydrolysis products during their formation. Denaturation by shearing is a common drawback of cellulase enzymes (Reese and Robbins, 1981), especially at air–liquid interfaces (Kim et al., 1982). Stabilizing agents such as surfactants are effective in reducing the extent of this deactivation.

Enzymatic hydrolysis processes are of interest because enzymes catalyze only specific reactions. Therefore, unlike acid hydrolysis, there are no side reactions or by-products and the hydrolysis can potentially be run at yields approaching 100% of theoretical. All enzymatic hydrolysis processes consist of four major steps that may be combined in a variety of ways – pretreatment, enzyme production, hydrolysis, and fermentation. Shielding of the cellulosic surface by lignin, crystallinity, and the inaccessibility of the cellulose to the enzymes are possible barriers to enzymatic attack.

4.4.4.1 Mechanism of Enzymatic Hydrolysis

A number of comprehensive reviews have been published regarding the mode of attack by cellulases on crystalline cellulose (Ghose and Ghosh, 1978; Bisaria and Ghose, 1981; Chang et al., 1981). The specific theories which have arisen since Reese's original concept (Reese et al., 1950) are quite diverse (Eriksson, 1969; Reese, 1977; Wood, 1980) but the underlying basis seems to be the same. The mechanism involves the cleavage of internal glycosidic bonds by *endo*-glucanase followed by the synergistic attack of *endo*- and *exo*-glucanases. Final hydrolysis of the product oligosaccharides is catalyzed by β-glucosidase. This sequential association between *endo*- and *exo*-glucanase has been demonstrated with the use of electron microscopy by White and Brown (1981).

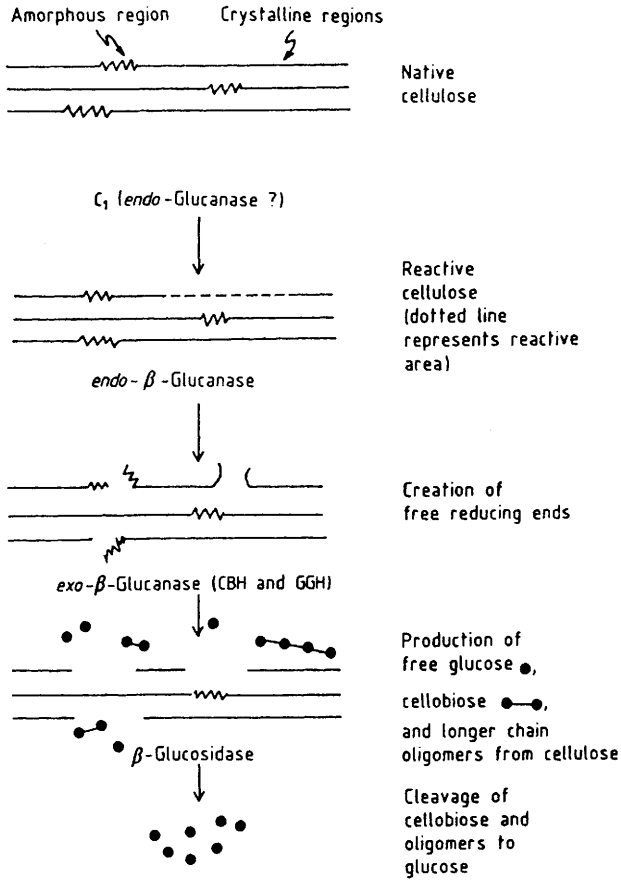

Fig. 16. Schematic representation of the synergistic action of enzymes involved in cellulose degradation. Note the role of C_1 in crystalline swelling (Bisaria and Ghose, 1981).
CBH: cellobiohydrolase; CGH: glucanase glucohydrolase.

Reese (1977) has speculated on the existence of a special member (C_1) of the endo-glucanase component (C_x). This enzyme is not only capable of splitting covalent glycosidic linkages but also of swelling crystalline cellulose by the disruption of hydrogen bonds. Fig. 16 schematically illustrates the synergistic nature of these components.

4.4.4.2 Comparison of Enzymatic and Acid Hydrolysis

Chemical or biochemical degradation of cellulosic substrates may be compared in terms of a number of properties inherent in each process as summarized in Tab. 16.

Tab. 16. Comparison of Enzymatic and Acid Hydrolysis Processes for Cellulosic Materials

Acid	Enzyme
1. Non-specific catalyst, therefore will delignify material as well as hydrolyze cellulose	Specific macromolecular catalyst, therefore extensive physical and chemical pretreatment of material necessary to make cellulose available for degradation
2. Decomposition of hemicellulose to inhibitory compounds (i.e., furfural)	Production of clear sugar syrup ready for subsequent anaerobic fermentation
3. Harsh reaction conditions necessary and, therefore, increased costs for heat- and corrosion-resistant equipment	Run under mild conditions (50 °C, atmospheric pressure, pH 4.8)
4. High chemical costs require catalyst recovery and reuse	Cost to produce cellulases is the most expensive step in the process, therefore, recycle is necessary
5. Rate of hydrolysis is high	Lower rate of hydrolysis
6. Overall yield of glucose is low due to degradation	High glucose yield depending upon system and pretreatment

5 Fermentation Modes of Industrial Interest

Ethanol can be produced by applying mainly four types of operations in industry; batch, continuous, fed-batch and semi-continuous. Batch and continuous production of ethanol are more widely used.

Cell recycle may advantageously be used with any of these operations, but the substrate must be essentially free of insoluble materials which could accumulate with the cell during centrifugation and build up to unacceptable amounts in the system. For example, sugar juices, molasses, and clear starch hydrolyzates are suitable whereas whole ground grain or mandioca roots are not (Keim, 1983).

All of the systems used in industry have some advantages with some disadvantages. The type of the process should be chosen regarding the properties of raw material used, and the investment and operating costs. The productivity and yield values are also important in deciding the system. The process which requires the minimum capital costs with maximum product recovery is wanted in industrial fermentations.

5.1 Batch Process

Today, most ethanol is produced by the same processes developed in the beverage industry more than a hundred years ago. These methods are based on the simple batch fermentation of carbohydrate feedstocks. In batch fermentation, substrate and separately grown cell slurry are charged into the bioreactor together with nutrients and enzymes required (Keim, 1983).

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The general characteristics of batch systems are well known. Usually the time required to completely utilize the substrate is 36–48 h. The temperature is held at 10–30 °C and initial pH is adjusted to 4.5. Depending upon the nature of the carbohydrate material, conversion efficiency lies in the range of 90–95% of the theoretical value with a final ethanol concentration of 10–16% (w/v). There is a specific reaction period for the cultivation. During this time, cell, substrates (carbon source, nutrient salts, vitamins, etc.) and product concentrations alter. This rapid increase slows down towards the end of the cultivation period with the rate approaching zero asymptotically. After as long as 50–60 h, the fermented material is pumped to distillation supply tank. Then, the bioreactor is washed, sterilized, and recharged with the new batch. The time lost in emptying, cleaning, and filling reduces the effective reactor volume by about 20%. Batch operation, however, has some advantages:

- Low investment costs are required with not much control.
- Low requirement for complete sterilization.
- Use of unskilled labor.
- Low risk of financial loss.
- Easy management of feedstocks.
- Greater flexibility is achieved by using a bioreactor for various products specifications.
- Well-defined cultivation periods are possible, so higher conversion levels are obtained.
- Less risk of infection and cell mutation, since relatively short cultivation periods are used.

However, inherent disadvantages of this system exist, such as:

- Non-productive idle time for emptying, cleaning, sterilizing, cooling, heating, and recharging the bioreactor, so only 80% of the reactor is effective.
- Frequent sterilization results in drifts of measuring instruments.
- Preparing several subcultures for inoculum and the control of this non-stationary process requires more expenditure.
- Greater risk to service personnel from possible contact with some pathogenic microorganisms or toxic products.
- Initial growth lag decrease the reactor productivity while microbes are locked in the exponential phase of their growth cycle in continuous processes.

These disadvantages have led to interest in variations of the process.

In spite of these disadvantages batch operations are preferred when:

- Only small amounts of products are considered.
- One reactor is enough for producing various products.
- There is a high risk of infection.

- There is a risk of mutation of microorganisms.
- The product is separated from the bioreactor discontinuously.

In an effort to increase fermenter productivity yet retain the simplicity of a batch process, cell recycle has been employed in many cases. This technique does not increase the efficiency of sugar-to-ethanol conversion, however, the time required for the fermentation to run to completion is reduced by as much as 60–70% over traditional batch methods.

The sugar solution supplemented with yeast nutrients is added to the fermenter and the fermenter is inoculated with a rapidly growing culture of yeast from the seed tank. A maximum in ethanol productivity is reached after 14–20 h. Ethanol production then continues at a decreasing rate until about 95% of the sugar is utilized.

Usually several fermenters are operated at staggered intervals to provide a continuous feed to the distillation system. The overall productivity for this process is about 1.8–2.5 kg ethanol produced per m^3 fermenter volume per hour (Rose, 1976). To increase the efficiency of the fermentation step, e.g., the "Melle Boinot process" is utilized in most Brazilian distilleries. This process involves centrifugal recuperation of the live yeast from the fermented beer (normally 10–15% by volume of the total) and reinoculating to other fermenters. Tab. 17 presents data for alcohol produced from annexed and autonomous distilleries. Fig. 17 illustrates the steps required to produce ethanol from cane juice molasses in Brazil.

5.2 Fed-Batch Processes

Fed-batch operation which can be regarded as a combination of batch and continuous operations is a very popular type of process in ethanol industry. In this

Tab. 17. Productivity Factors for Ethanol Production from Sugarcane (Lindeman and Rocchiccioli, 1979)

Productivity Factor	Alcohol Indirectly from Final Molasses	Alcohol Directly from Sugarcane Juice
Sugarcane yield in 1.5-2 year cycle (south-central region)	63 t ha^{-1}	63 t ha^{-1}
Average sucrose yield (13.2 wt.%)	8.32 t ha^{-1}	8.32 t ha^{-1}
Crystal sugar production	7.0 t ha^{-1}	–
Final molasses and cane juice production	2.21 t ha^{-1}	66.2 t ha^{-1}
Fermentable sugars, molasses, and juices	1.32 t ha^{-1}	8.73 t ha^{-1}
Alcohol yield at 100% global efficiency	675 kg ha^{-1}	4,460 kg ha^{-1}
Alcohol yield with reasonable 85% global efficiency	11.5 L t^{-1} cane or 730 L ha^{-1}	75 L t^{-1} cane or 4,800 L ha^{-1}

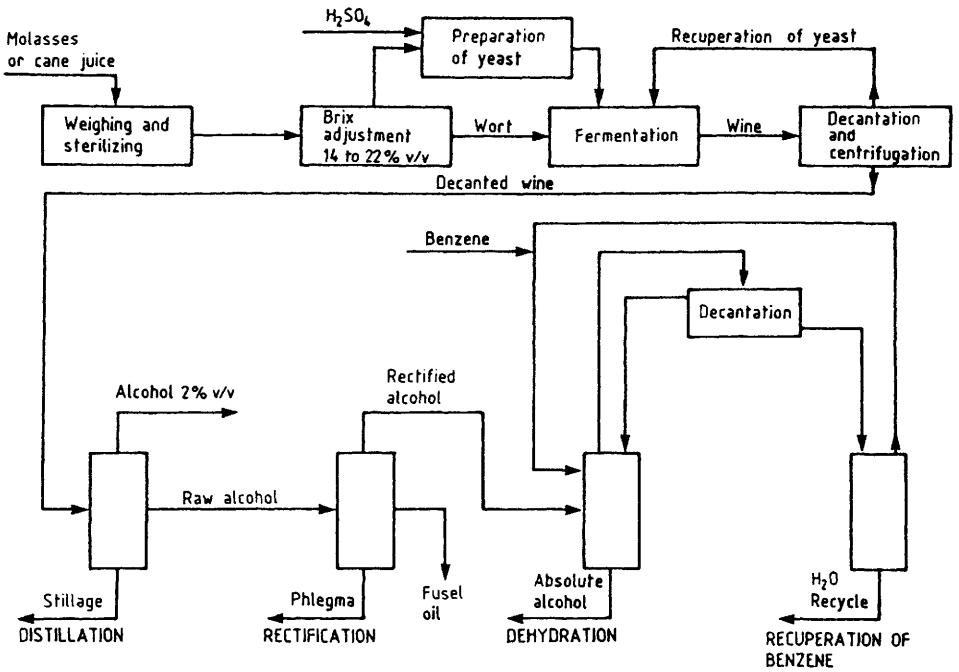

Fig. 17. Block diagram for ethanol distillery (Lindeman and Rocchiccioli, 1979).

operation, feed solution which contains substrate, yeast culture, required minerals and vitamins are fed at constant intervals while effluent is removed discontinuously. The main advantage of fed-batch system is that substrate inhibition or catabolite repression are prevented by intermittent feeding of the substrate. If the substrate has an inhibitory effect, intermittent addition of the substrate improves the productivity of the fermentation by maintaining the substrate concentration low. The start-up of fed-batch operation is similar to the batch process start-up. Subsequently, substrate is fed to the reactor in a specified manner, after the growth-limiting substrate (generally carbon source) which is given at the beginning of the process has been consumed. The concentration of the substrate should be kept constant in the reactor while the feeding is made. In this way, substrate inhibition can be kept at a minimum level in fed-batch processes by adding substrate at the same rate at which it is consumed. Substrate concentration can be measured and feed controlled accordingly, so the levels can be kept low. The substrate consumption rate is calculated from measured factors such as carbon dioxide production rate, when substrate concentration cannot be measured directly. The process is continued until another nutrient becomes limiting and/or an inhibitory concentration of product is reached.

The advantages of fed-batch operation are:

- High yield is achieved since well-defined cultivation time (no cells are added or taken away during the cultivation period) is possible in fed-batch operation. Hence, the reactor operates batchwise as far as the microorganisms are concerned.
- High level of flexibility.
- Semi-stationary method of operation even in the case of slightly mutating microorganisms and those involving an infection risk.
- Optimization of the environmental conditions such as the growth or production phase and the age of the culture of the microorganisms is possible.

The disadvantages of fed-batch operation are:

- Non-productive idle time is required for filling, heating, sterilizing, cooling, emptying, and cleaning the reactor.
- Higher manpower requirements or expensive instruments such as process computers are required (e.g., to keep the substrate concentration requires expensive instruments).
- Greater possible risk to service personnel of contact with pathogenic microorganisms or toxic products.
- More wear and tear on instruments from frequent sterilization.

In spite of these disadvantages, fed-batch processes are often practiced when continuous methods are impossible (e.g., due to slight mutation or infection of microorganisms) and batch operation would result in low productivity values.

A fuzzy-decision-making procedure was applied to find the optimal feed policy of a fed-batch fermentation process for fuel ethanol production using a genetically engineered *Saccharomyces* yeast 1400 (pLNH33) (Wang et al., 1998). The policy consisted of varying feed flow rate, feed concentration, and fermentation time. The recombinant yeast 1400 (pLNH33) could utilize glucose and xylose simultaneously to produce ethanol. However, the parent yeast utilizes glucose only. A partially selective model was used to describe the kinetic behavior of the process. In this study, this partially selective fermentation process was formulated as a general multiple-objective optimal control problem. By using an assigned membership function for each of the objectives, the general multiple-objective optimization problem could be converted into a maximizing decision problem.

5.3 Semi-Continuous Processes

The semi-continuous processes (Yarovenko, 1978) comprise the so-called outflow–inflow and overflow processes and also a wide range of battery and cyclic fermentation variants.

In semi-continuous processes a portion of the culture is withdrawn at intervals and fresh medium is added to the system. Repeated fed-batch culture, which can be maintained indefinitely, is another name of the semi-continuous process. Characteristic of these processes is the continuous feed liquid flow after addition of a nutritive wort and of a seeding culture, such as *Saccharomyces cerevisiae*. The treated medium flows by gravity or is pumped as a seeding culture from the first vessel into the second, while fermentation is continued in the first. Then, in succession, the second fermenter is filled up with the nutritive wort and left for fermentation. Thereafter, the third vessel is filled with the seeding culture and a prolonged nutritive inflow charge is secured. This process continues until all the vessels are charged.

It is essential to maintain the culture volume constant in continuous operation whereas there is volume variation in semi-continuous processes. Semi-continuous operation is used for some industrial fermentations including production of vinegar, penicillin, baker's yeast and waste disposal by fermentation. This method has some of the advantages of continuous and batch operations:

- There is no need for a separate inoculum vessel, except at the initial start-up.
- Time is not wasted in non-productive idle time for cleaning and re-sterilization.
- High flexibility of operation.
- Less wear and tear on instruments from sterilization.
- Not much control is required.

Using this system, the fermentation time is shortened because of higher yeast activity and concentration. It also has some disadvantages:

- Slightly higher investment costs are required due to larger reactor volumes.
- High risk of contamination and mutation due to long cultivation periods and periodic handling.
- Complications exist resulting from the combined biomass and product formation stages.

In spite of these disadvantages semi-continuous operation is used in industrial ethanol production. Semi-continuous processes can also be used under a vacuum. In the semi-continuous vacuum operation, fresh medium is continually fed to the bioreactor in order to maintain a constant volume as ethanol and water is boiled away. A bleed stream of fermented broth is not removed from the bioreactor. However, components in the medium which are not metabolized by the yeast also accumulate in the bioreactor under this mode of operation (Cysewski, 1976).

A novel repeated-batch operation method was devised for ethanol fermentation using the flash fermenter system (Ishida and Shimizu, 1996). In this method, the fermenter beer was exchanged at intervals between the fermenter with biomass recycle and the distillation unit, thereby promoting the selective

removal of ethanol. Experimental data were used to develop a mathematical model. High performance was attained, with a productivity of about 12 g L^{-1} h^{-1} and a product concentration of 400 g L^{-1}. The simulated results suggested that higher product concentrations could be obtained by increasing the batch period of operation (t) and that the value of the fraction of the overhead liquid taken as the product divided by t should be decreased. Substrate conversion efficiency or yield were not taken into account in the study.

5.4 Continuous Processes

Continuous ethanol production eliminates much of the unproductive down-time associated with batch culture. This includes stripping and cleaning the apparatus, recharging with media, and time required for the lag phase. Feed, which contains substrate, culture medium and the other required nutrients is pumped continuously into an agitated vessel where the microorganisms are active. The sugar is largely consumed and ethanol and new cell mass are produced during the process. The product, which is taken from the top of the bioreactor contains ethanol, cells, and residual sugar. Air is sparged through the bioreactor in order to maintain the cell growth. The composition of the solution in the bioreactor is assumed uniform and the same as the composition of the overflow stream. However, throughput must be constant if the steady state is involved. In the bioreactor, new cells are continuously born, but cells are also continuously washed out. A steady state is achieved when the growth and washout rates are identical. A cell density of only 10–12 g L^{-1} is typical. The overall productivity for a simple C.S.T.R. using a high productivity yeast is approximately 6 g L^{-1} h^{-1} ethanol which is three times the average batch productivity (Maiorella et al., 1981).

In addition, since the microorganism may be essentially "locked" in the exponential phase of its growth cycle, then the overall time-dependent productivity of ethanol formation is increased. This allows for a higher output per unit volume of equipment and as such, cost savings may be made in the construction of smaller fermenters (Maiorella et al., 1981).

The advantages of continuous operation are:

- Mechanization and automation are made for large scales.
- Continuous process requires lower manpower.
- Lower reactor volumes, as there are no unproductive down-time taken up with emptying, filling, and sterilization of the reactor.
- Since operating conditions are invariant, product quality is constant.
- Less possible danger to service personnel from pathogenic microorganisms or toxic materials, due to improved mechanization.
- Less wear and tear on instruments from sterilization.

In spite of these many advantages, there are disadvantages in continuous systems, such as:

- Raw material quality must be uniform as operating conditions cannot be adapted so easily.
- Problems in maintaining a high fermentation rate.
- Low flexibility is reached since only slight changes on throughput, medium composition, temperature, oxygen concentration are possible.
- Continuous sterilization of the medium, using expensive control and automation equipment cause mainly high investment costs.
- High risk of microorganism mutation due to long cultivation periods (either internal through mutation or external by an invading microbe).
- Continuous removal of non-soluble, solid substrates can be very expensive.

Low rates of fermentation have been shown to be connected with cell death caused by a lack of oxygen. This oxygen requirement may be eliminated by the addition of certain substances (i.e., Tween 80, ergosterol, or linolenic acid) to the fermentation medium or by propagation of the yeast under aeration prior to anaerobiosis (Beran, 1966).

Specific ethanol productivity in a simple continuous fermenter is ordinarily limited by ethanol inhibition and a low cell concentration. As sugar content of the feed stream is increased, ethanol productivity decreases due to product inhibition effects. At lower concentrations of carbohydrate, inhibition is seen to decrease, however, cell mass concentration also falls off. Thus, an optimum fermenter productivity is reached at about a 10% glucose feed as seen in Fig. 18.

In view of all the advantages and disadvantages, continuous operation is preferred for processes with high production rates, for gas, liquid or soluble

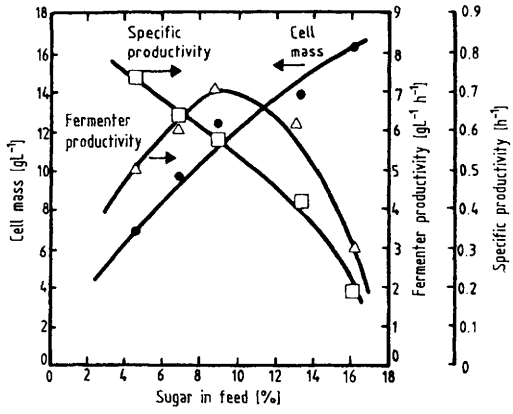

Fig. 18. Effect of glucose concentration on continuous fermentation; conditions at "complete" substrate utilization (Cysewski and Wilke, 1978).

solid substrates and when microorganisms with high mutation stability are involved.

Cell recycle has been utilized in continuous systems to overcome low cell density limitations. With much of the microbial biomass returned to the fermenter an extremely high cell concentration may be maintained. To retain cell viability, a fraction of biomass is removed on a continuous basis. Densities as high as 83 g L^{-1} may be maintained in the fermenter for such a system (Del Rosario et al., 1979) and productivities of up to 30–50 g L^{-1} h^{-1} ethanol are possible.

Complexities arise due to the requirement for some separation device in this process. Mechanical centrifuges increase capital costs and require substantial maintenance. Other methods have been developed to simplify the cell concentration step such as settling tanks (Wash and Bungay, 1979) with the possible addition of agents which will enhance flocculation (Weeks et al., 1982).

A multistage continuous ethanol fermentation from molasses with recycle of the yeast is used by the Danish Distilleries Ltd. of Grena (Rosen, 1978). Fig. 19 shows the process. The received molasses is stored in 2–3 tanks, each approximately 1,500 m^3, and is then pumped to intermediate containers. From there, the raw material is taken to a set of metering pumps, for molasses, water, H_2SO_4, and $(NH_4)_2HPO_4$. The pH is adjusted to 5. This mixture is sterilized in

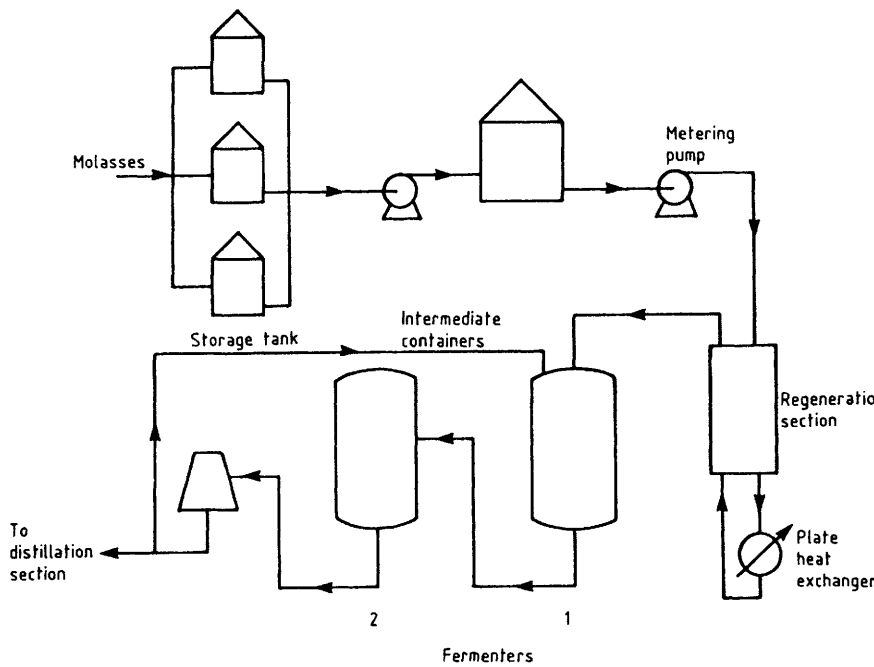

Fig. 19. Continuous production of ethanol by the Danish Distilleries Ltd. (Rosen, 1978).

a plate heat exchanger at 100 °C. Two or three fermenters are used, with a total volume of about 170 m^3. The fermented wort is centrifuged prior to distillation and the yeast returned to fermenter 1.

When the fermentation is started, molasses, water, and ordinary baker's yeast are added to fermenter 1, which is aerated until the yeast has propagated sufficiently to reach the total quantity desired. Approximately 0.02–0.03 L of air per L of liquid per min has to be infused.

Characteristic fermentation data are as shown in Tab. 18. The yield calculated on molasses amounts to approximately 28.29 L alcohol per 100 kg molasses, or a maximum of approximately 65 L per 100 kg fermentable sugar.

Alcon Biotechnology Ltd. (Alcon, 1980) have developed a rapid continuous fermentation process for ethanol production. This process operates with any clean fermentable sugar feedstock including sucrose from sugarcane, sugar beet, and molasses and any hydrolyzed starch from such sources as corn, sorghum, and cassava. A simplified flow sheet is shown in Fig. 20. The process uses a single stirred tank fermenter in which yeast is maintained at a high concentration (up to 45 g L^{-1}) by recycling.

The fermenter is stirred either by liquid recirculation or by a conventional agitator. The temperature and pH are controlled automatically. The fermenter is equipped with dosing systems for the addition of antifoam, alkali, and nutrients. The oxygen level is controlled. Yeast is separated by gravity settling. For clarified beet juice at sugar concentrations of 14% (w/v) the ethanol yield is about 93% of the theoretical value at dilution rates of 0.14–0.18 h^{-1}. The ethanol concentration in the outlet was 8.5–8.9% (w/v). The capital cost of this system should be low as only one fermenter and gravity settling are used.

A plant using continuous tower fermenters has been under consideration in Australia (Anonymous, 1980a). Cereal, molasses, cane juice, sugar beet, cassava, or coarse grains are being considered as substrates.

The fermenter consists of a vertical cylindrical tower with a conical bottom. The tower is topped by a large-diameter yeast settling zone fitted with baffles. The sugar solution is dumped into the base of the tower which contains a plug of flocculent yeast. Reaction proceeds progressively as the beer rises, but yeast tends to settle back and be retained. High cell densities of 50–80 g L^{-1} are

Tab. 18. Performance Data for the Danish Distilleries Process (Rosen, 1978)

Component	Fermenter 1[a]	Fermenter 2
Yeast, dry matter [g L^{-1}]	10	10
pH	4.7	4.8
Alcohol [vol.%]	6.1	8.4
Residual sugar [%]	1.0	0.1
Temperature [°C]	35	35

[a] Residence time in each fermenter: 10.5 h; afflux: 600 kg molasses diluted in $22 \cdot 10^3$ L h^{-1}.

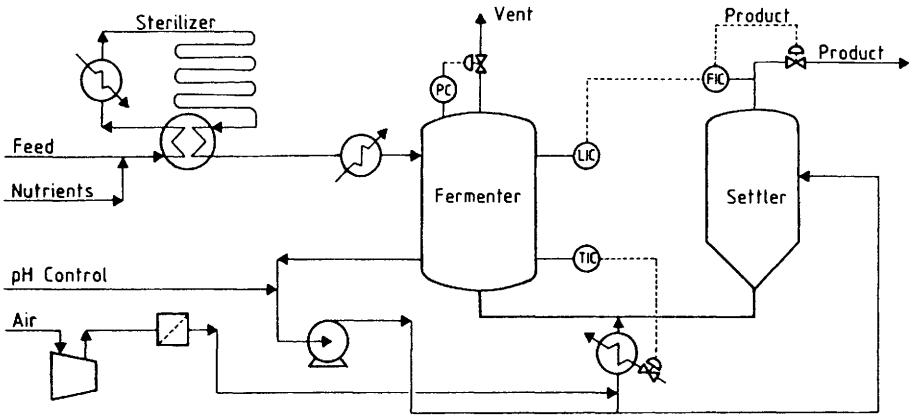

Fig. 20. Flowsheet of the ALCON continuous fermentation process.
FIC: flow indicating controller; LIC: level indicating controller; TIC: temperature indicating controller; PC: pressure controller.

achieved without an auxiliary mechanical separator. Residence times of below 4 h have been possible with sugar concentrations of up to 12% (w/v) sucrose giving 90% sugar utilization and 90% conversion to ethanol, i.e., up to 5% (w/v) alcohol.

Productivities of up to 80 times higher than for a simple batch process have been achieved in the tower fermenter. Some apparent difficulty is associated with providing the desired oxygen concentration in the fermenter. A major drawback of this APV Co. Ltd. system is the long time required for initial start-up. Two or three weeks are required to build up the desired high cell density and achieve stable operation. This is compensated by the very long (more than 12 months) operating times between shutdowns. Capital and operating costs are predicted to be far lower than for conventional batch processes.

6 Industrial Processes

6.1 Types of Bioreactors for Ethanol Production

Various types of bioreactors are being used in industrial ethanol production. One of them is series C.S.T.R.s. Advantages have been demonstrated for this type of bioreactors arranged in series both at laboratory and production scale. Systems with several reactors in series can also be considered. The concept of multiple series of C.S.T.R.s has been applied in the multistage perforated plate column bioreactor which is shown in Fig. 21. Even though an increase in productivity and reduction in overall volume is obtained, the mechanical complex-

ity of the perforated plate column reactor is a major factor against its industrial application.

Bioreactors operated with cell recycle are another group of reactors. Cell recycle has been employed in many cases in order to increase reactor productivity and concentration of biomass (Melzoch et al., 1991). The use of continuous cell recycle reactors has been thoroughly investigated and applied at large scale. A cell recycle system is shown in Fig. 22.

The system is identical to the simple C.S.T.R.s except that a centrifuge is used to separate yeast from the product overflow and this yeast is returned to the reactor (Kosaric et al., 1983). With continued cell growth, and cell escape prevented, the cell concentration in the reactor becomes extremely high and total productivity is greatly increased.

Complexities arise due to the requirement for some separation device in this process. Mechanical centrifuges increase capital costs and require considerable maintenance. Electrical energy costs are increased, and added supervision is required to monitor the centrifuge. These disadvantages have been shown to be more than offset by the great increase in productivity and reduction in equipment size.

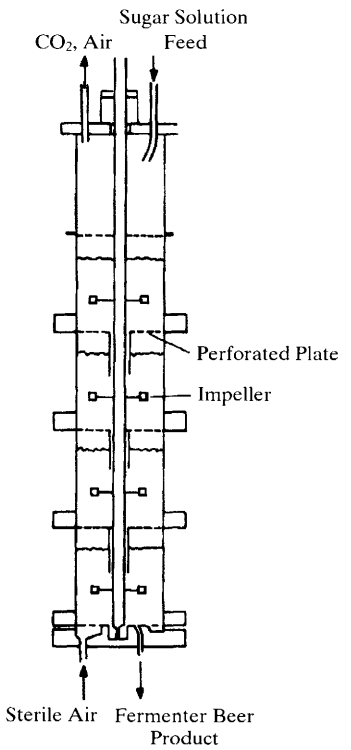

Fig. 21. Multistage perforated plate column fermenter.

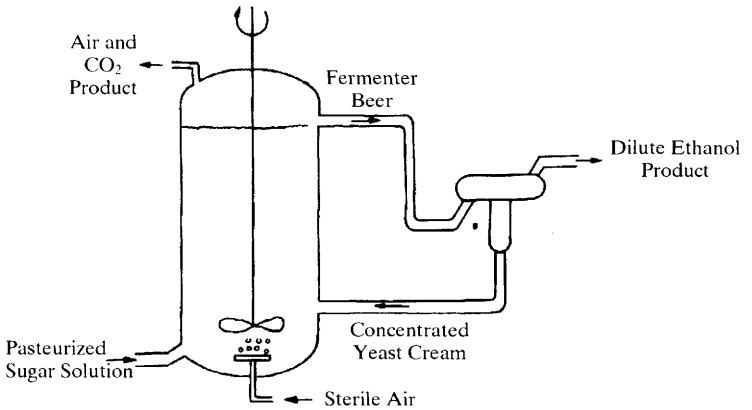

Fig. 22. Continuous stirred tank reactor with yeast recycle.

Several attempts have been made to develop simplified cell recycle systems which do not require mechanical centrifuges. Simple cell settling systems have been proposed where the cells are thermally shocked, and allowed to settle by gravity. Very large settling tanks are required in this system. Whirlpool separators have been investigated, which is shown in Fig. 23.

Another type of fermenter which is called partial recycle reactor has been developed and tested at pilot scale (Fig. 24). The separation is far from complete, but some concentration of cells is achieved, and with essentially no added equipment.

Another group of bioreactors applied is tower bioreactors for which many designs have been tested. One of them, the APV tower system (Fig. 25) has been operated successfully at large scale.

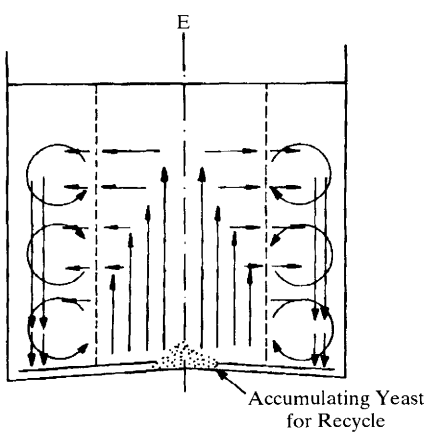

Fig. 23. Whirlpool yeast separator.

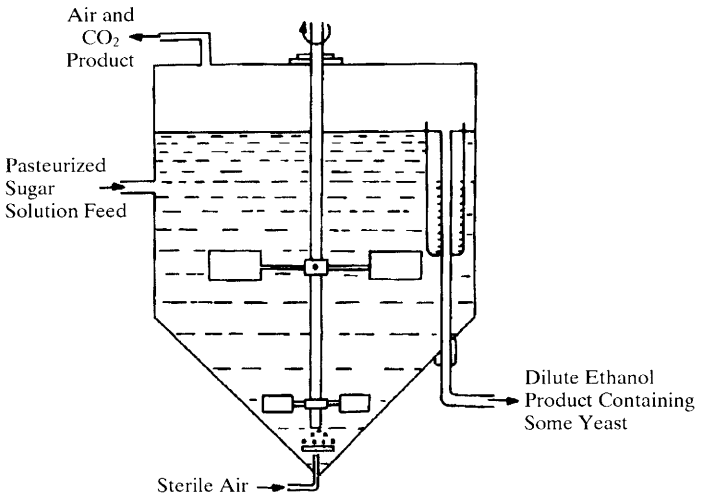

Fig. 24. Partial recycle reactor.

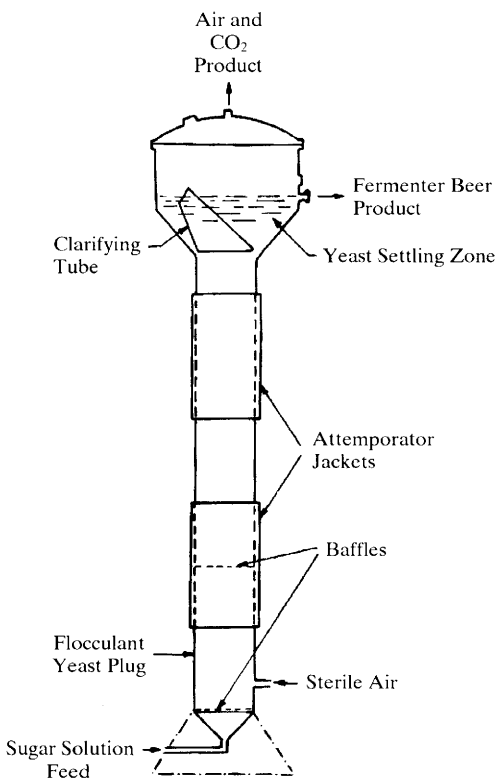

Fig. 25. APV tower fermantor.

The main advantage of tower bioreactor is that high cell densities are achieved without the requirements of auxiliary mechanical separator. Productivities 32 to 80 times those for simple batch processes have been achieved with the APV tower system. Different yeasts and sugar feed solutions have been used for the various systems. A major disadvantage of the APV tower system is the long time required for initial start-up. Building up the desired high cell density and achieving stable operation require two to three weeks. This is compensated by the very long run times between shutdowns.

In bioprocess industry, tubular reactors are also very powerful devices, as they realize narrow residence time distribution. Industrial application of plug flow bioreactors is stimulated by a renaissance of continuous culture techniques, e.g., bulk products like ethanol. Plug flow reactors have been in application for a long time sterilization, enzyme technology, and partly in wastewater treatment together with some other special fields of application (Paar et al., 1987). However, the vertical reactors often are preferred to the horizontal ones. Horizontal reactors – compared to vertical tubes (tower reactors) – have some disadvantages such as lower oxygen efficiency and gas residence time as the height of towers can be greater than the diameter of horizontal tubes, but offer also some advantages (plug flow behavior is not influenced by CO_2 evolution and pressure is not necessarily high).

Another type of bioreactor which is used in some industrial applications is fluidized-bed bioreactor. Fluidized-bed bioreactors are often operated with immobilized cell systems. The application of these cell systems to ethanol production offers clear advantages of bioconversions in fluidized-bed reactors. These advantages are:

- High cell densities and high yields are achieved.
- Prevention of washout is possible.
- Control of operation is easy.
- Operation with low hydraulic residence time and low risk of contamination is possible.

Fluidized-bed reactors have also some advantages, such as the high turbulence created in the fluid–solid mixture. This leads to much higher heat transfer coefficients than those which can be obtained in the other types of bioreactors. Therefore, fluidized-bed reactors have also found use in exothermic processes where close temperature control is important.

The design and operation of fluidized-bed bioreactors is by no means an easy task, especially when the recirculation of solids is involved. There is much more technological operation knowledge involved than with packed beds.

The use of packed-bed bioreactors for bioconversions using immobilized cells or enzymes has the advantage of simplicity of operation, high mass transfer rates, and high volumetric reaction rates (for processes which are not inhib-

ited by the substrate concentration). High biomass loadings are achieved by immobilizing the cells; hence washout, which is a problem of several systems, can also be prevented (Hamamci and Çolak, 1991). However, the immobilization materials, gels, are somewhat compressible and the production of CO_2 causes the beads to be compressed and the void fraction to decrease. The result is a high pressure drop and phase separation together with a decrease in ethanol productivity.

Packed-bed cylindrical bioreactors have some disadvantages in use such as an increase in bed surface area in the direction of flow resulting in a drop in fluid velocity and a reduction in the pressure drop (Matteau, 1987). One approach to successfully circumvent these disadvantages has been the development of two novel packed-bed bioreactor geometries, which are known as a radial-flow packed-bed, and a tapered packed-bed reactor.

The plug bioreactor, however, is another approach (Fig. 26). Using the plug bioreactor, productivities 72 times greater than for simple batch with the same yeast and substrate have been obtained (Maiorella et al., 1981). The requirement of high pressure equipment will partly offset the capital cost savings resulting from reduced vessel volume. High power pumps will be required to force medium through the plug bioreactor, and the required shutdowns for yeast regeneration will interrupt continuous operation. Further study is also

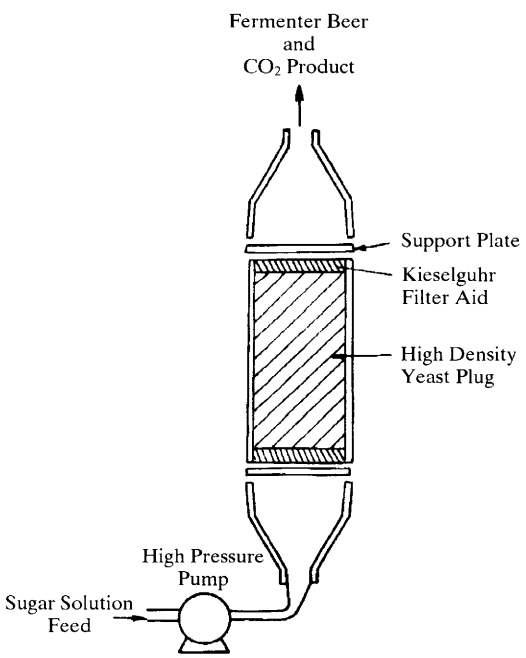

Fig. 26. High cell density plug fermenter.

necessary to determine if these disadvantages are offset by the very high possible productivities.

6.1.1 Solid Phase Fermentation (Ex-Ferm Process)

A somewhat innovative process for ethanol production was developed in Guatemala (Rolz, 1978, 1979, 1981) with the objective of reducing costs. It employs fresh or dried sugarcane pieces as a raw substrate. It is a mixed solid–liquid phase system where sucrose extraction and fermentation are conducted simultaneously. Liquid from the first fermenter (packed-bed fermenter) is usually used in a second cycle of extraction–fermentation. This could be repeated until ethanol tolerance is reached or until microbial contamination is too high. A more complete juice extraction is claimed (2.2 L per 103 kg) and no separate extraction equipment but modifications of the fermenter are required.

6.1.2 Simultaneous Saccharification and Fermentation (SSF) Process

The simultaneous saccharification and fermentation (SSF) process for ethanol production was conceptualized in the late 1970s by Gauss et al. (1976), Takagi et al. (1977), and Blotkamp et al. (1978). Employing fermentative microorganisms in combination with cellulose enzymes, sugar accumulation in the fermenter is minimized. In this way, feedback inhibition by product sugars is reduced and higher hydrolysis rates and yields are possible than for saccharification without fermentation at high substrate loadings. Wright et al. (1988) presented results combined with a process model to evaluate the economics of the process and to investigate the effect of alternative processes, conditions and organisms.

Spindler et al. (1989, 1991) evaluated 4 woody crops, 3 herbaceous crops, corn cobs, corn stover, and wheat straw in the SSF process as substrates pretreated with dilute acid for ethanol production by *Saccharomyces cerevisiae* and *Brettanomyces clausenii*. In all cases, SSFs demonstrated faster rates and higher conversion yields than the saccharification without fermentation (SACs) processes.

In this process, increased yield is favored by long residence times, low substrate and product concentrations, and high enzyme loadings. Product concentration, on the other hand, is favored by low yields, high enzyme loadings, and long residence times. Thus, there is a trade off between yield, product concentration, and enzyme consumption and the optimum combination of these parameters should be determined.

The commercial production of ethanol from cellulose by SSF is prevented in part by the high cost of fungal cellulase enzymes. Intermittent exposure of SSF

processes to ultrasonic energy under selected conditions (5 FPU (filter paper units) of cellulase per g of substrate; 15 min of exposure per 240 min cycle during the latter half of SSF) was found to increase ethanol production from mixed waste office paper by approximately 20%, producing 36.6 g L^{-1} ethanol after 96 h (70% of the maximum theoretical yield). Without ultrasound, 10 FPU of cellulase per g of substrate was required to achieve similar results. Continuous exposure of the organism to ultrasonic energy was bacteriostatic and decreased ethanol production but may be useful for controlling bacterial growth in other processes (Wood et al., 1997).

A verified mathematical model was used to examine the most critical biochemical engineering aspects of ethanol production by Philippidis and Hatzis (1997). Extensive simulations of the SSF of cellulose were conducted to identify the effects of operating conditions, pretreatment effectiveness, microorganism parameters, and enzyme characteristics on ethanol production. The results clearly showed that the biomass–enzyme interaction plays a dominant role in determining the performance of SSF in batch and continuous operating modes. In particular, the digestibility of the substrate (as a result of pretreatment) and the cellulase enzyme dosage, specific activity, and composition had a profound effect on ethanol yield. This investigation verified the conclusion that R & D emphasis should be placed on developing more effective pretreatment methods and producing cellulase preparations of high specific activity (low cost per enzyme unit) to realize gains from any development of advanced hexose/pentose-fermenting organisms

Kadam and Newman (1997) evaluated several industrially available nutrient sources for their effectiveness in the SSF of pretreated poplar with *Saccharomyces cerevisiae* D5A, in order to develop a cost-effective fermentation medium. The nutrients that were evaluated were corn steep liquor (CSL), urea, $MgSO_4 \times 7H_2O$, and $(NH_4)_2SO_4$. It was concluded that a suitable low-cost medium that exhibited good ethanol yields and productivities was a combination of 0.3% CSL and 2.5 mM $MgSO_4 \times 7H_2O$. This medium was approximately 50 times less expensive than a nutrient-rich medium. Furthermore, this alternative medium consists of components that are available on a commercial scale and is, hence, more industrially relevant than the rich medium

In another study (Brooks and Ingram, 1995), a recombinant strain of *Klebsiella oxytoca* was tested with commercial fungal cellulase in optimized SSF experiments using unsorted, mixed waste office paper (MWOP) as a substrate (pH 5–5.2 and 35 °C). This organism ferments cellobiose and cellotriose to ethanol at near theoretical yields, eliminating the need for supplemental β-glucosidase. Similar rates and yields were obtained with dilute acid-pulped (hydrolysis of hemicellulose) and water-pulped MWOP on a dry weight basis although viscosity was reduced by the acid pretreatment. In simple batch fermentations, 40 g L^{-1} ethanol was produced after 48–72 h with 100 g L^{-1} MWOP and 1,000 FPU of cellulase L^{-1}, a yield of 550 L of ethanol per metric ton. Cellu-

lase usage was further reduced by recycling SSF residues containing bound enzymes in multistage fermentations. This approach reduced the requirement for fungal cellulase while retaining rapid ethanol production and high ethanol yield. In the optimal design, broths containing an average of 39.6 g L^{-1} ethanol were produced in three successive stages with an average fermentation time of 80 h (567 FPU of fungal cellulase L^{-1}; 6.1 FPU per g of substrate). This represents a yield of 0.426 g of ethanol per g of substrate, 539 L per metric ton, 129 U.S.gal per ton. MWOP contains approximately 90% carbohydrate. Thus the combined efficiency for saccharification and fermentation to ethanol was 83.3% of the theoretical maximum.

6.1.3 Recycle Systems

Biomass and stillage recycle was used to modify the conventional ethanol fermentation process, thereby providing a more economical process. The method of biomass recycle resulted in a reduction in the usage of raw material by approximately 8%, whereas the method of stillage recycle achieved less water consumption and a reduction in stillage volume (Shojaosadati et al., 1996). Details of processes developed in Germany are presented by Senn and Pieper in the present book.

Recycling the contents of a continuous fermenter through a stripping column has been proposed as a means of reducing product inhibition and lowering the cost of fuel ethanol production (Taylor et al., 1995). A 2-L fermenter and 10-cm packed column were continuously operated for 150 d without contamination. Some fouling of the packing with attached yeast cells was observed which partially blocked the column. Cell yield was lower than in a simple continuous fermenter. Complete conversion of 200 g L^{-1} glucose feed and 90% conversion of 600 g L^{-1} glucose feed were achieved. Data were analyzed by computerized process simulation. Cost analysis indicated that, with heat recovery to reduce heating and cooling costs, the continuous fermenter/stripper is possibly a lower-cost alternative to conventional fermentation and distillation.

6.1.4 Novel Reactors for On-Line Product Removal

As alcohol is toxic to cell growth, processes by which it may be removed upon formation would greatly enhance the productivity of the system. Novel reactors have been developed for on-line product removal in order to achieve high productivities by maintaining high cell densities.

The vacuum bioreactors provide an alternative with continuous ethanol production. The system is shown in Fig. 27. Cysewski and Wilke (1977) have taken advantage of the high volatility of ethanol by running the fermentation under

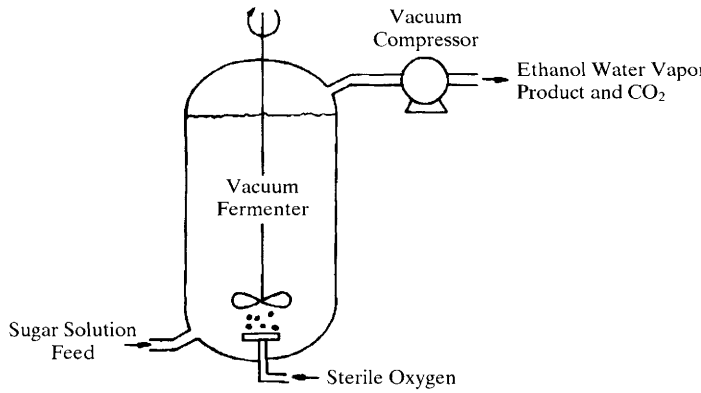

Fig. 27. Continuous vacuum fermentation.

sufficient vacuum to boil off the product at temperatures conductive to yeast growth. A fermenter productivity of 82 g L^{-1} h^{-1} was achieved from a 33.4% (w/v) glucose feed (12-fold that of a conventional continuous process).

Energy requirements for the vacuum fermentation process are increased only by 5% over those for conventional processes, when suitable techniques for energy recovery are employed. The absolute productivity achieved in the vacuum bioreactor is higher than has yet been achieved in other devices and this advantage offsets the small increase in energy requirements for the vacuum system.

The flash fermentation process modifies the simple vacuum fermentation system to overcome some of the operating difficulties of vacuum bioreactor. The flash system is shown in Fig. 28.

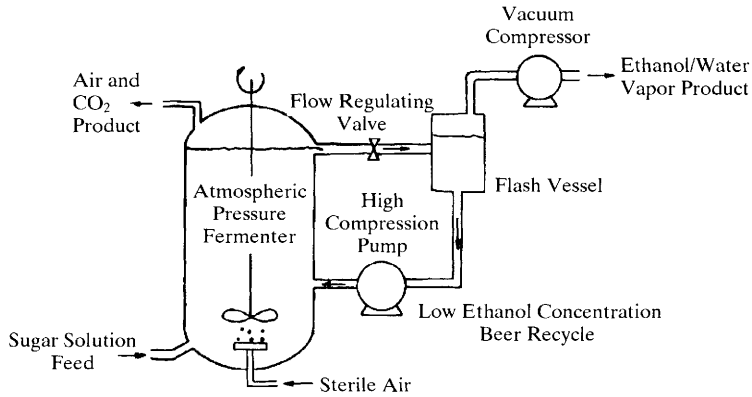

Fig. 28. Continuous flash fermentation.

In the extractive process, which is a new technique developed for continuous operation, ethanol is continuously removed from the reaction mixture by solvent extraction (Fig. 29). This leads to high reaction rates by eliminating ethanol inhibition (Maiorella et al., 1981).

Gyamerah and Glover (1996) describe the fermentation production of ethanol in a continuous pilot plant, with liquid–liquid extraction being used to remove the product and with recycle of the fermented broth raffinate. The pilot plant was operated for up to 18 d, and feed glucose concentrations ranged from 10.0–45.8% (w/w). The rate of ethanol production was not adversely affected by the concentration of by-products in the fermented broth. A mathematical model predicting the time needed for achievement of 99% of the steady-state by-products concentrations was in good agreement with the experimentally determined concentration of glycerol, the dominant by-product (Fig. 30).

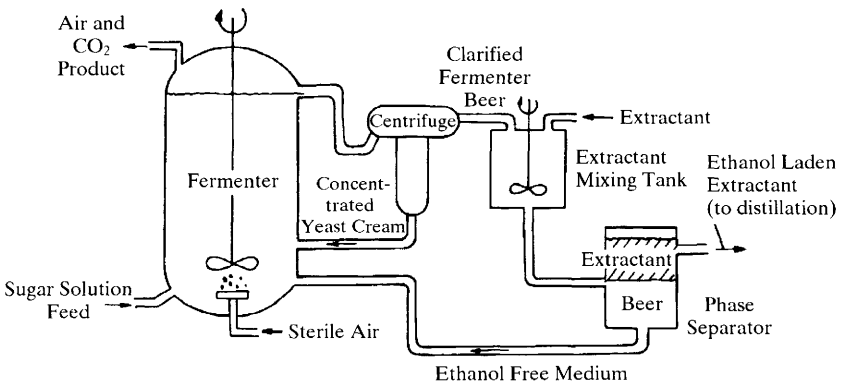

Fig. 29. Continuous solvent extraction fermenter.

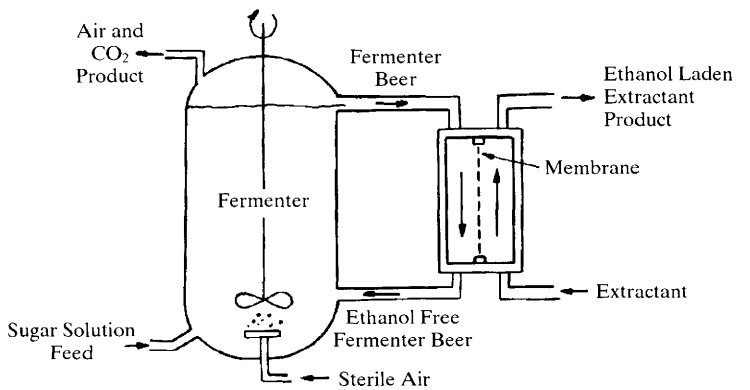

Fig. 30. Product recovery membrane bioreactor.

In order to achieve a successful process, an extractant must have these properties:

- non-toxic to yeast,
- high distribution coefficient for ethanol,
- selective for ethanol over water and secondary fermentation products,
- should not form emulsions with fermentation broth.

If in addition to the required properties, the extractant is of low volatility, distillation will be simplified and considerable energy savings could result over conventional systems. In terms of the process evaluation criteria, extractive fermentation, with a modest increase in complexity, yields a very high productivity in a low-energy system. However, the main disadvantage of this system is that no extractant possesses all the required properties.

Continuous ethanol recovery from the reaction mixture can also be achieved using selective membrane separation techniques. Membrane extractive fermentation (perstraction), which is shown in Fig. 30, is similar to the simple extractive process except that the extractant is separated from the broth by a diffusion membrane (Maiorella et al., 1981).

In the membrane extractive process, requirements for the extractant are far less severe than in direct extractive fermentation. This is a simple process which involves little added equipment and may allow an energy reduction in distillation. Fouling of the membrane and frequent shutdowns for membrane replacement is costly and thus a major drawback. In spite of this disadvantage, membrane perstraction is used widely in industry.

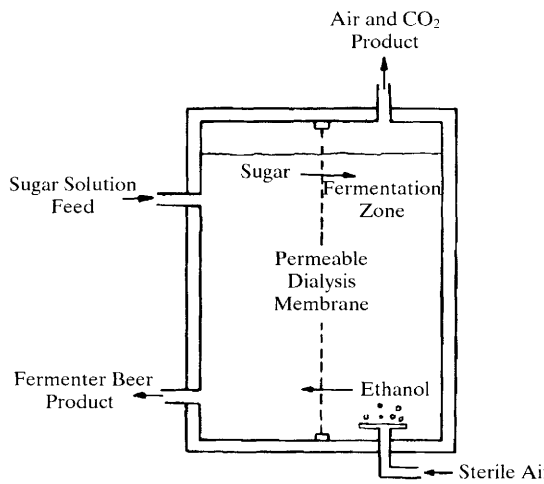

Fig. 31. Simple continuous membrane fermenter.

Selective product recovery membrane fermentation is another new technique under development. The apparatus used in this type of membrane fermentation is like that used for membrane extractive fermentation except that no extractant is used in selective product recovery membrane fermentation. The membrane itself performs the separation, facilitating ethanol diffusion through the membrane while retarding water and other beer components.

A variety of reactor designs, most of which may be classified as either stirred vessels with recycle or hollow fiber units, exist. The membrane bioreactor enables reduction of the product inhibitory effect in consequence of its partial and continuous removal from the fermentation zone in addition to the effective immobilization of cells in the bioreactor and maintenance of their metabolic activity.

In the simple continuous membrane system which is shown in Fig. 31, extremely high cell densities can be achieved since cells cannot escape the reaction zone (Maiorella et al., 1981). For this system, reaction rate is limited by the rate at which substrate can diffuse across the limited membrane surface area. This practical diffusion limitation prevents very rapid reaction in a simple system.

The limitations of low substrate diffusion rate, which is another problem of a membrane bioreactor, can be prevented by using the pressure membrane which is shown in Fig. 32. However, this system has a disadvantage since free proteins (the product of cell lysis) rapidly pile up on the membrane pores and prevent further flow (Maiorella et al., 1981).

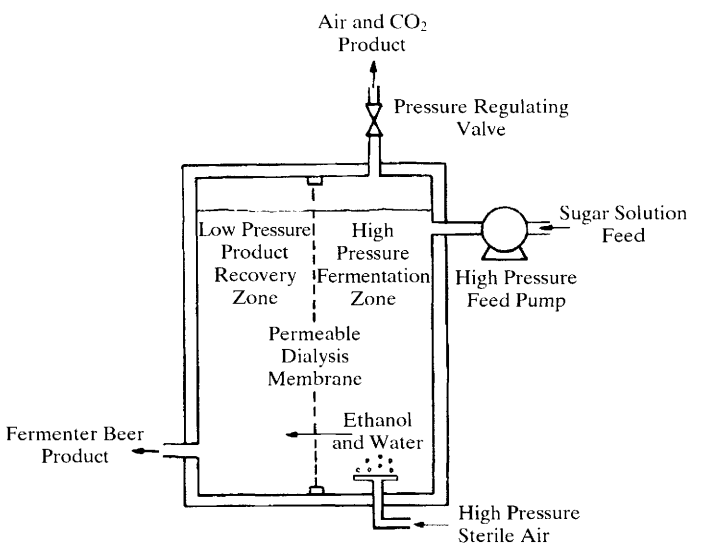

Fig. 32. Pressure membrane fermenter.

The problem of membrane fouling is overcome if the rotorfermenter is used (Maiorella et al., 1981). The rotorfermenter is a continuous pressure membrane bioreactor (Fig. 33). The rotorfermenter is advantageous if high productivity is aimed. However, the rotating membrane unit is mechanically complicated and thus is expensive (Maiorella et al., 1981).

A multimembrane reactor is a different bioreactor design, in which microporous membranes separate the reactor into gas, immobilized cell, nutrient, and extractant layers. This arrangement of compartments within the reactor differs from other membrane-based bioreactor systems. The multimembrane bioreactor concept is quite flexible and applicable to any of a variety of fermentations (Shuler and Steinmeyer, 1990).

Another approach to achieving high rate membrane process involves the use of hollow fiber reactors, which is shown in Fig. 34.

High cell densities and high ethanol productivities are possible in the hollow fiber bioreactor (Mulder and Smolders, 1986). However, venting of carbon dioxide gas product and membrane plugging are disadvantages of this system together with their complexity and cost.

From a process point of view, the pervaporate flux and the selectivity are the key parameters in designing a process. The flux can be increased by decreasing the membrane thickness or by increasing the feed temperature. However transport limitation of vapors at the down-stream side may occur at very high

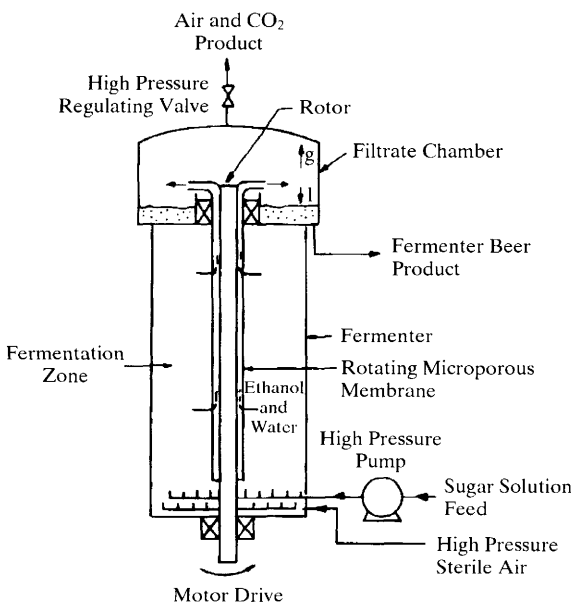

Fig. 33. Rotor fermenter.

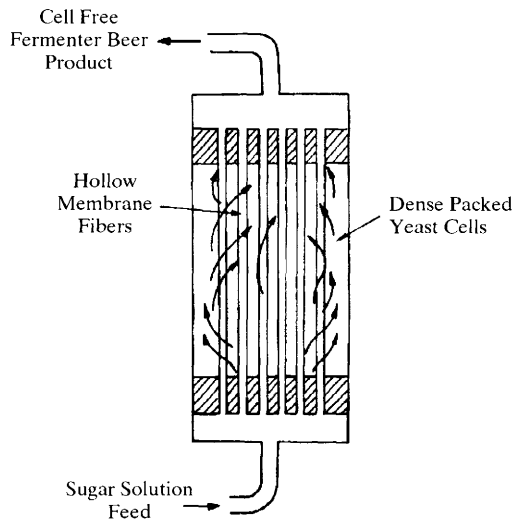

Fig. 34. Hollow fiber fermenter.

fluxes (Groot et al., 1991). Membrane materials that have a higher selectivity than pure silicone rubber have already been reported, e.g., silicone copolymers. The addition of silicalite, a hydrophobic zeolite, to silicone rubber can lead to a higher selectivity.

The different advanced high rate processes described in the preceding section have been assessed as to their potential for application in industrial alcohol production. The comparisons made are summarized in Tab. 19. Many alternatives are superior to the conventional batch technology. Among these, simple continuous, series continuous, cell recycle and tower bioreactors have been operated at large scale with considerable savings over batch processes.

Among the on-line ethanol removal processes which eliminate end product inhibition, only vacuum and flash fermentation have been advanced sufficiently to allow pilot plant testing. The other high rate processes require much more evaluation before their merits can be fully assessed (Maiorella et al., 1981).

6.2 Some Examples of Industrial Processes

6.2.1 Ethanol from Corn

A flow diagram for a conventional fermentation plant producing 76.0×10^3 m^3 a^{-1} anhydrous ethanol from 816.5×10^3 kg d^{-1} corn is shown in Fig. 35.

Corn from storage is fed to a grinder where the kernel size is reduced to expose the interior portion of the grain. Water is added, pH adjusted, and the

Tab. 19. Alternative Production Processes (Maiorella et al., 1981)

Process	Ethanol Productivity	Comments
Batch processes Simple CSTR bioreactor	very low [1.8–2.5 g L^{-1} h^{-1}] low [6 g L^{-1} h^{-1}]	very high capital and operating cost mechanically simple equipment, simple continuous operation
Series CSTR bioreactors	2–3 times rate for simple	simple continuous operation
Continuous cell recycle bioreactors	CSTR high	settlers appear to be too large for industrial application. Whirlpool separators may be attractive
Tower bioreactors	high	mechanically simple with simple continuous operation, but start-up period is very long
Packed-bed bioreactors	high	plugging and by-passing are major problems
Plug bioreactor	high (72 times greater than for similar batch processes)	frequent shutdowns
Vacuum fermentation	very high [80 g L^{-1} h^{-1}]	mechanically complicated equipment requiring constant monitoring. Small added energy requirements for vapor compression. Contamination of the vacuum vessel may be a problem. Pure oxygen must be sparged
Extractive fermentation	potentially very high	no suitable extractant has been found. If membrane extractive process is used membrane fouling may be a problem, process is otherwise simple
Dialysis bioreactor	not reported but potentially high	reaction rate limited by substrate diffusion through membrane and membrane fouling will be a major problem in pressure dialysis bioreactor
Rotor bioreactor	high [36 g L^{-1} h^{-1}]	membrane fouling problem overcome but the bioreactor is mechanically quite complex. Membrane destruction by mechanical shear may be a problem
Hollow fiber	not reported but potentially high	expensive units. Mass transfer limitation may limit productivity

ground grain is then cooked to solubilize and gelatinize the starch. After cooking and partial solubilization, fungal amylase is added. Yeast, which has also been propagated in the plant, is added and the fermentation is allowed to continue for approximately 48 h at a temperature of 32 °C. During this time about 90% of the original starch in the grain is converted to alcohol. The processing proceeds on a cyclic or batch basis, with some vessels containing fermenting mash while other vessels are being filled, emptied, or sterilized.

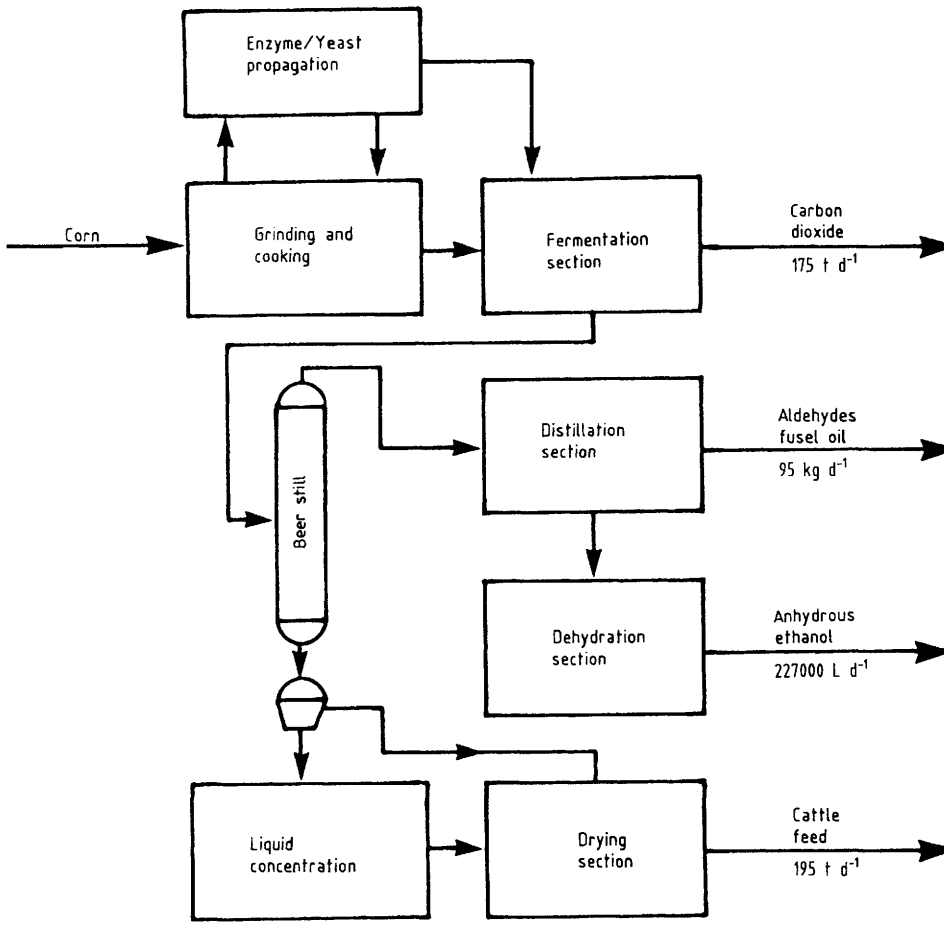

Fig. 35. Proposed flow diagram for a conventional fermentation plant producing 76 mio L of anhydrous ethanol per year from corn (Scheller, 1976).

Once fermentation is completed, the mixture is fed to the beer still where essentially all of the alcohol is distilled overhead to about 50 vol.%. The diluted alcohol is purified by further distillation, which removes fermentation by-products (aldehydes, ketones, fusel oils), yielding 95 vol.% alcohol. If it is desired to produce anhydrous ethanol, the 95 vol.% ethanol is fed to a dehydration section consisting of an extractive distillation with benzene.

In the beer still, the remaining water with dissolved and undissolved solids is drawn from the bottom of the still and fed to a centrifuge. The liquid phase from the centrifuge is concentrated to 50% dissolved solids in a multiple-effect evaporator and mixed with the solids from the centrifuge. This mixture is then dried in a fluidized transport-type dryer to 10% moisture and is used as cattle

feed. The cattle feed contains all the proteins that were originally present in the grain, plus the additional proteins from the yeast, resulting in a product containing 28–36% protein by weight.

In addition to alcohol and cattle feed, the original 816.5×10^3 kg of corn yield 175.0×10^3 kg of carbon dioxide and 95 kg of by-product aldehydes, ketones, and fusel oils.

Ethanol production can be incorporated into a wet milling corn processing plant. In this case, the substrate is the isolated corn starch and the by-product is gluten feed.

6.2.2 Ethanol from Cassava Root

The first commercial plant in the world producing ethanol from cassava root commenced production in 1977, with an ethanol capacity of 60 $m^3\ d^{-1}$ (Anonymous, 1978). It is expected that additional plants will be built in the future using similar processing.

The processing steps used to obtain ethanol from cassava roots are shown in Fig. 36. As the fresh roots are received, they are weighed, washed, peeled, and ground into a mash. Part of this mash, the quantity determined by the plant operating plans, is then side-streamed and dried, producing a meal. Unlike sugarcane, which starts to decay and ferment naturally soon after cutting, dried cassava can be stored for as long as one year without a significant loss of starch, as well as bitter cassava. Bitter cassava varieties contain cyanogenic glucosides which do not cause any trouble as they are flashed off with the steam during the mash cooking process (Jackson, 1976).

The starch molecules are broken down with the aid of α-amylase which is added in two steps. The preliminary addition is required to decrease the viscosity of the mash and so facilitate the cooking operation. Glucoamylase is then employed to achieve the final conversion of the liquefied starch material into glucose. This is the time limiting step in the overall process. The final steps in the process of recovering the alcohol from the fermented material are the same as those for sugarcane juice.

Alcohol yields from cassava are in the range of 165–180 L t^{-1}. However, as sugarcane harvests can be up to 90 t ha^{-1}, the alcohol yield per unit cultivation area is higher for sugarcane under present agricultural methods. Also, the greatest advantage from sugarcane processing is in the use of its fiber (bagasse) as a fuel. A cane stalk contains roughly the same weight of dry fiber as sugars and, therefore, is more than adequate in providing the energy for alcohol processing. This is not the case with cassava, where the dry fiber content is about 3.5%. Also, because of the necessary conversion of starch to fermentable sugars, a greater energy input is necessary in its processing prior to fermentation.

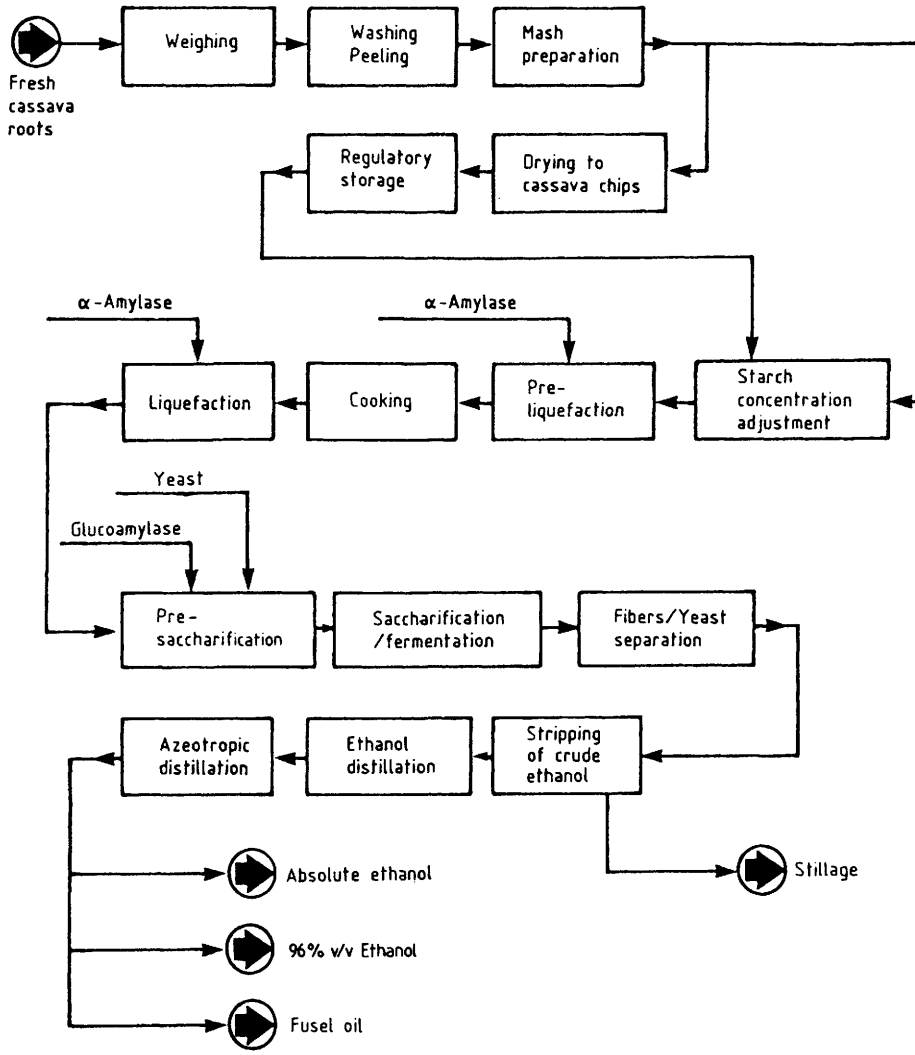

Fig. 36. Production of ethanol from cassava root (Lindeman and Rocchiccioli, 1979).

For batch alcohol manufacture from cassava, the steam requirement is about 1.8 kg steam per kg cassava, which must come from an external source. By continuous processing in which efficient heat recovery systems are used, a considerable fuel reduction can be achieved. Therefore, the biggest problem in using cassava is the high energy requirement.

6.2.3 Ethanol from Potatoes

The Danish Distilleries Ltd. of Aalborg has developed a semi-continuous process for the production of ethanol from potatoes and grain (Rosen, 1978). In this process (Fig. 37), the potatoes (or grain) are transported on a conveyor belt to a belt weigher.

Comminution of the potatoes or grain and the blending of grain and water take place in a pulper. The enzyme used is Termamyl 60 prepared by Novo Industri. After enzymatic treatment, the potatoes or grain are heated to 90–95 °C by flash steam in a condenser. The raw material is then transported from the bottom of this apparatus to a boiler tube which is approximately 50 m in length. Heating to a temperature of approximately 150 °C is achieved by 10.1 MPa steam. Retention time at 150 °C is approximately 3 min and the rate of speed in the boiler tube is around 0.3 m s^{-1}. The mixture is flashed to atmospheric pressure in a single step, and the steam released is used to preheat the starchy compound. The mash is cooled to 70 °C for liquefaction with commercial amylase preparations of bacterial origin. If pH regulation is required, it is accomplished with slaked lime. Intermission at 70 °C is approximately 25 min. The mash is cooled to 30 °C and is then pumped to the yeast vessels, where batch fermentation to ethanol is carried out in a conventional manner. All temperature and level regulations are automatic, so that the process can be maintained by one operator.

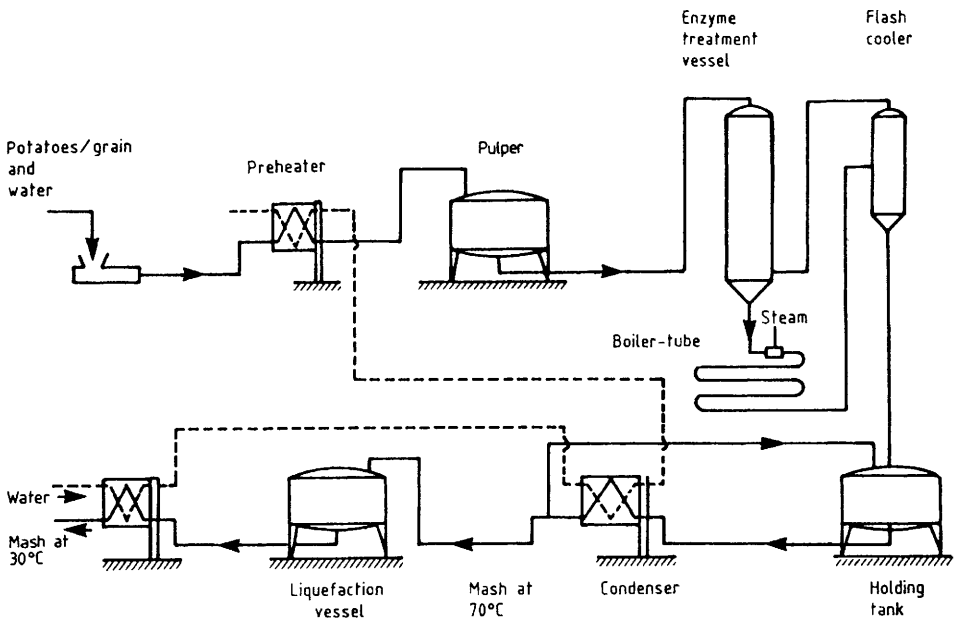

Fig. 37. Danish Distilleries Ltd.'s semicontinuous production of alcohol from potatoes/grains (Rosen, 1978).

6.2.4 Ethanol from Jerusalem Artichoke Tubers (Topinambur)

A process to produce 360×10^3 kg a^{-1} ethanol from Jerusalem artichoke tubers was designed by Kosaric et al. (1982). A simplified flow sheet of the process is shown in Fig. 38.

The juice from the ground tubers is obtained by two-step expression with macerating. The final juice contains approximately 12–15% of carbohydrates. The juice is hydrolyzed enzymatically by maintaining it at 56 °C for 2 h at pH 3.8. There is enough enzyme present in the plant, particularly if harvested in spring. After cooling, the juice is fermented in the batch mode with recycle of the yeast. The ethanol yield of about 90% is achieved after 28 h. Complete hydrolysis is not required as a special strain of yeast with inulase activity is used.

6.2.5 Ethanol from Carob Pod Extract

Roukas (1994) studied continuous ethanol production from carob pod extract by immobilized *Saccharomyces cerevisiae* in a packed-bed reactor. When nonsterilized carob pod extract containing 200 g L^{-1} total sugars was used as feed material, the maximum ethanol productivity achieved was 24.5 g L^{-1} h^{-1} at a dilution rate of 0.5 h^{-1}. This represented a theoretical ethanol yield of 58.8% and sugars' utilization of 85%. The bioreactor system was operated for 30 d at a constant dilution rate of 0.5 h^{-1}. No loss of the original immobilized yeast ac-

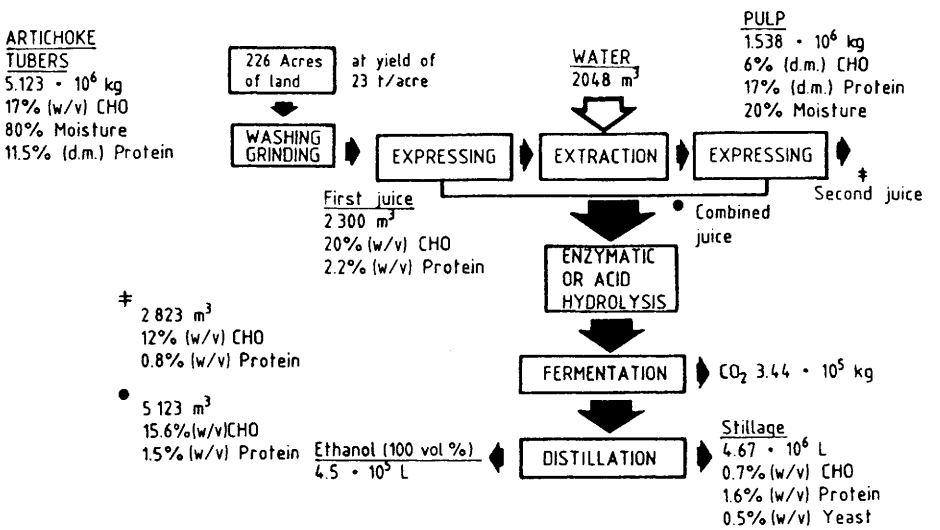

Fig. 38. Flowsheet for production of 4.5×10^5 L ethanol (100 vol.%) per year from Jerusalem artichoke tubers (Kosaric et al., 1982).

tivity was found. The high ethanol productivity obtained at relatively high sugars' utilization and the excellent bioreactor stability show that the process of continuous production of ethanol from carob pod extract by immobilized *S. cerevisiae* is very promising.

6.2.6 Ethanol from Cellulose

Ethanol from cellulosic biomass is a promising renewable liquid transportation fuel. Applied research in the area of biomass conversion to ethanol in the last 20 years has answered most of the major challenges on the road to commercialization but, as with any new technology, there is still room for performance improvement.

6.2.6.1 Dilute Sulfuric Acid Process

As mentioned earlier, dilute sulfuric acid hydrolysis of wood was re-examined by the Forest Products Laboratory, U.S. Department of Agriculture, at the request of the War Production Board in 1943. A full-scale wood hydrolysis plant was built in Springfield, Oregon, but it did not commence operation until after the end of World War II (Hokanson and Katzen, 1978). A schematic diagram of the plant is shown in Fig. 39. The process is basically a semi-continuous process in which the hydrolyzate percolates through the "chip" bed, which avoids prolonged exposure of sugars to acid at high temperatures and so reduces degradation. Optimum conditions are listed in Tab. 20.

Dilute hydrolysis solution from a previous batch is pumped into the top of the hydrolyzer containing the wood wastes. After the dilute hydrolyzate is charged, hot water and sulfuric acid are added and the temperature is raised to 196 °C (1,520 kPa vessel pressure). After 70 min of pumping, a strong hydrolysis solution starts to flow out of the bottom of the hydrolyzer. Two letdown flash stages are used, the first stage operating at 456.0 kPa and the second stage

Tab. 20. Optimum Conditions for Ethanol Production from Wood Hydrolyzate (Hokanson and Katzen, 1978)

Parameter	Optimum Conditions
Acid concentration in total water	0.53%
Maximum temperature of percolation	196 °C
Rate of temperature rise	4 °C min^{-1}
Percolation time	145–190 min
Ratio of total water-oven-dried wood	10
Percolation rate	8.69–14.44 L min^{-1} m^{-3}

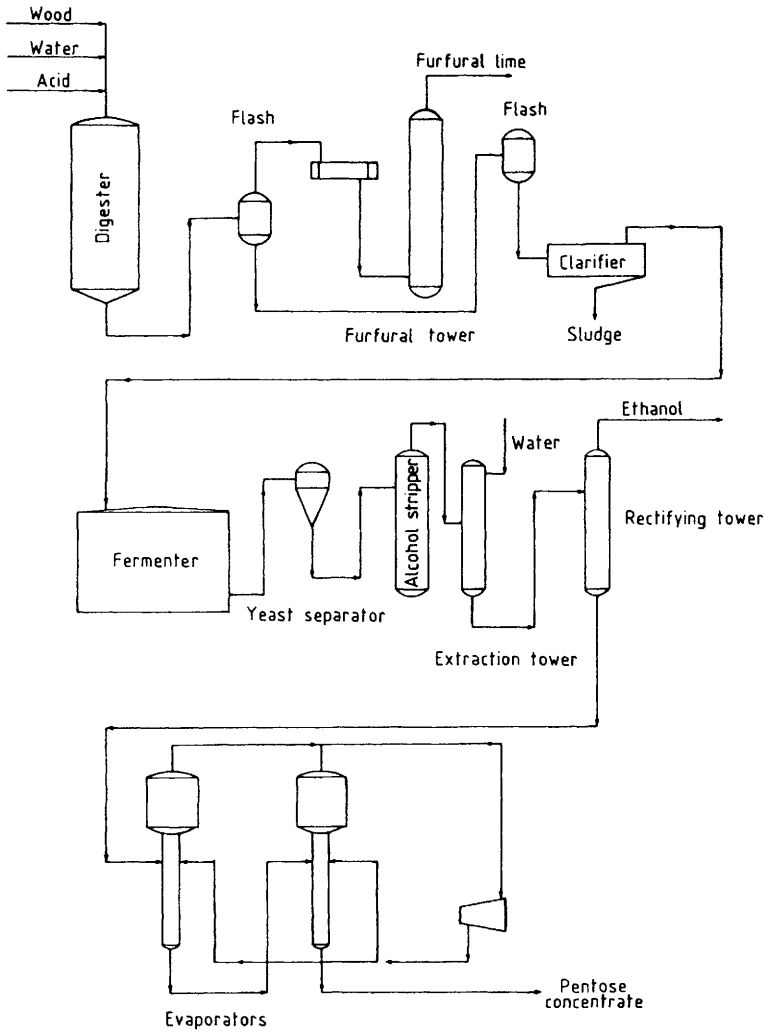

Fig. 39. Ethanol production from wood (Hokanson and Katzen, 1978).

at atmospheric pressure. The condensates from the flash vapor from both stages contain furfural and methanol, which are recovered. The underflow from the two flash stages is the sugar-containing solution. At the end of the percolation cycle, the lignin-rich residue is discharged, recovered, and used as a fuel.

The flash condensate passes to a distillation tower for recovery of methanol. From the base of this tower the bottoms pass to a second distillation tower for recovery of the furfural–water azeotrope. The hot acid hydrolyzate solution is neutralized with a lime slurry and the precipitated calcium sulfate is separated in a clarifier. Calcium sulfate sludge is concentrated to about 50% solids and is

trucked to a disposal area. Neutralized liquor is blended with recovered yeast from a previous fermentation and passed to fermentation tanks. From the fermenters, the fermented liquor passes to yeast separators for recovery of the yeast for recycling (*Saccharomyces cerevisiae*).

Ethanol is recovered in a series of distillation towers and is finally rectified to approximately 95 vol.%. Bottoms from the beer stripping tower contain pentoses. Instead of disposing of this stream, it is economically feasible to concentrate the sugars to a 65% solution for sale as a feed supplement or for conversion into furfural. The plant was closed for economic reasons after World War II.

Inventa AG (Switzerland) has developed a dilute acid hydrolysis process (Mendelsohn and Wettstein, 1981). The technology is based on the wood saccharification process operated until 1956 in Domat/Ems, which produced 10×10^6 kg a^{-1} of fuel-grade ethanol using locally available softwoods. Fig. 40 shows the flow sheet of this process (Mendelsohn and Wettstein, 1981).

The reactors operate on wood chips at about 1,000 kPa and at 140–180 °C. Hemicellulose and cellulose from wood are hydrolyzed with dilute (0.6 wt.%) acid. The solution of acid and sugars, the wort, is removed from the reactor and collected in a flash tank. The lignocellulose cake leaving the reactor is used as a fuel. The vaporized furfural is collected in a recovery section. The cooled wort is then neutralized with limestone ($CaCO_3$) and the gypsum is separated. The wort is cooled to the temperature required for fermentation and the nutrients are added. The fermentation takes place in a series of fermenters split into two parallel lines for operating flexibility.

A yeast adapted to wood hydrolyzates is used. The fermenters are stirred by gas (CO_2) recycled to the process. The mash with an ethanol concentration of

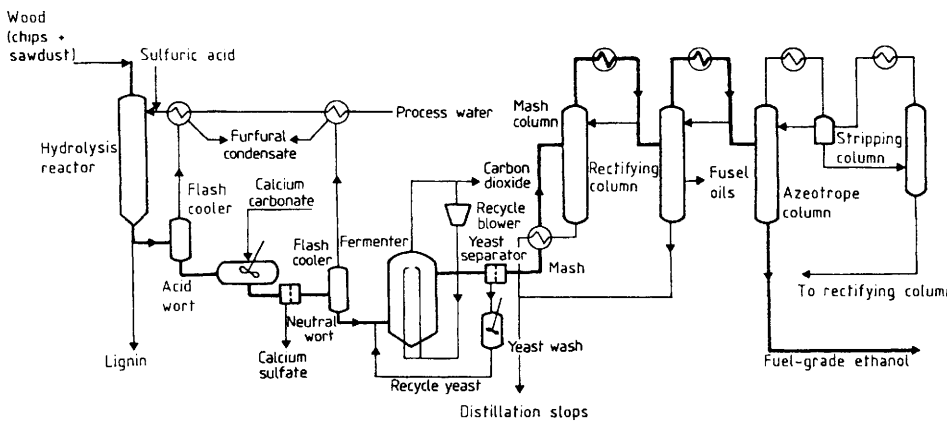

Fig. 40. Flowsheet for ethanol production by continuous fermentation (Mendelsohn and Wettstein, 1981).

about 2% is separated and a concentrated yeast suspension is recycled to the fermenter. Fuel-grade ethyl alcohol produced from the fermented wort is concentrated by distillation. Yield of alcohol is 240 L t^{-1} of wood dry matter. Steam consumption is $14–16 \times 10^3$ kg t^{-1} ethanol. The Inventa process can be energy-autonomous, with wood the only resource required.

6.2.6.2 Strong Acid Hydrolysis Process

For strong acid hydrolysis the proposal based on the Hokkaido Strong Acid Hydrolysis Pretreatment Process is worth mentioning.

The flowsheet in Fig. 41 shows the process. Undried wood is first introduced into a prehydrolysis digester column in which dilute sulfuric acid is used to remove the hemicellulose. Lignin cellulose particles then enter a pressurized feeder and are transported by recycling strong acid hydrolysis solution to the top of the second digester. In the second countercurrent column, the cellulose is hydrolyzed at room temperature by 70–80% sulfuric acid. The glucose–sulfuric acid solution leaving the top of the column is separated by electrodialysis membranes. The glucose retained by the membrane is neutralized and deionized before fermentation. The sulfuric acid permeated from the electrodialysis is evaporated and reconcentrated for recycle. Lignin is separated from the strong acid exiting the bottom of the second digester by filtration and washing.

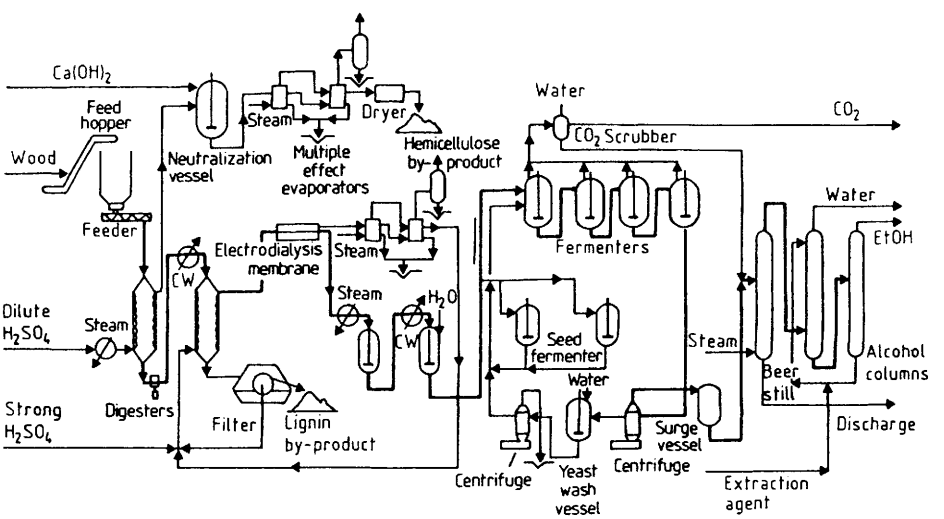

Fig. 41. Flowsheet for ethanol production from wood using the strong acid hydroysis method (Yu and Miller, 1980).

6.2.6.3 Ethanol Production from Agricultural Residues via Acid Hydrolysis

Two processes based on cornstalks have been employed (Sitton et al., 1979; Foutch et al., 1980): dilute acid treatment with sugar yields of up to 50% and concentrated acid treatment with even higher yields. The two-stage process involves a dilute sulfuric acid treatment for pentosan conversion followed by treatment with concentrated sulfuric acid for hexosan conversion. A high yield, low acid utilization, and no degradation of pentoses when contacted with concentrated acid occur.

Fig. 42 shows the flow diagram for the hydrolysis process and the mass balance for a plant with a capacity of 17×10^3 m^3 a^{-1} ethanol. Ground corn stover (20 mesh) is treated with 4.4% sulfuric acid at 100 °C for 50 min. The mixture is then filtered and the xylose-rich liquid is processed by electrodialysis for acid recovery. The solids are dried and impregnated with 85% H_2SO_4. Water is then added to the powder to form an 8% H_2SO_4 concentration, and hydrolysis is carried out at 110 °C for about 10 min. Acid is recovered from the glucose-rich stream by electrodialysis. The remaining solids can be dried and returned to the soil or used as a source of fuel. After hydrolysis xylose and glucose are obtained. The yield of xylose is 95% and the yield of glucose is 89%.

Glucose is converted to ethanol by *Saccharomyces cerevisiae* in the immobilized cell reactor. *Fusarium oxysporum* converts xylose to ethanol in another immobilized cell reactor. Cell overgrowth is removed every two weeks by sparging the reactors with compressed carbon dioxide. The ethanol in the reac-

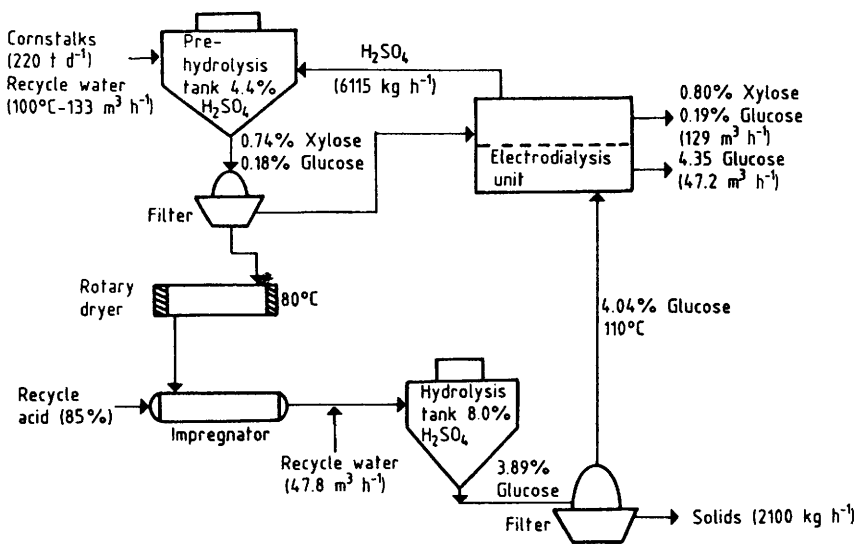

Fig. 42. Flowsheet for acid hydrolysis of cornstalks (Foutch et al., 1980).

tor effluent is separated by distillation. Fig. 43 shows the fermentation process for a plant with a capacity of 17×10^3 m^3 a^{-1}.

The process analyzed by Woodley et al., (1999b) can be briefly described as using co-current dilute acid prehydrolysis of the lignocellulosic biomass with simultaneous enzymatic saccharification of the remaining cellulose and co-fermentation of the resulting glucose and xylose to ethanol. In addition to these unit operations, the process involves feedstock handling and storage, product purification, wastewater treatment, enzyme production, lignin combustion, product storage, and other utilities.

The feedstock, in this case hardwood chips, is delivered to the feed handling for storage and size reduction. From there the biomass is conveyed to pretreatment and detoxification. In this area the biomass is treated with dilute sulfuric acid at a high temperature for a very short time, liberating the hemicellulose sugars and other compounds. Ion exchange and overliming is required to remove compounds liberated in the pretreatment that will be toxic to the fermenting organism. Detoxification is applied only to the liquid portion of the hydrolysis stream.

After detoxification, a portion of the hydrolyzate slurry is split off to enzyme production. In enzyme production, seed inoculum is grown in a series of progressively larger aerobic batch fermenters. The inoculum is then combined with additional hydrolyzate slurry and nutrients in large-production aerobic fermenters to produce the enzymes needed for saccharification.

Simultaneous saccharification and co-fermentation of the detoxified hydrolyzate slurry is carried out in a series of continuous anaerobic fermentation

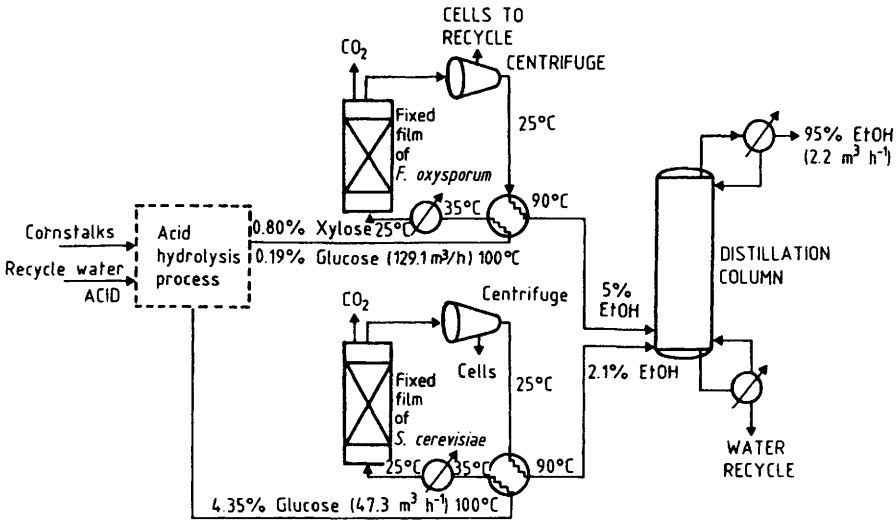

Fig. 43. Process for fermentation of hydrolyzate to ethanol (Foutch et al., 1980).

trains. The fermenting organism *Zymomonas mobilis* is grown in progressively larger batch anaerobic fermentations. The inoculum, along with cellulase enzyme and other nutrients, is added to the first fermenter. After several days of saccharification and fermentation, most of the cellulose and xylose will have been converted to ethanol. The resulting beer is sent to product recovery.

Product recovery consists of distilling the ethanol away from the water and residual solids. A mixture of nearly azeotropic water and ethanol is purified to pure ethanol using vapor-phase molecular sieves. Solids from the distillation bottoms are separated and sent to boiler. Concentration of the distillation bottoms liquid is performed by evaporation, using waste heat. The evaporated condensate is returned to the process as fairly clean condensate and the concentrated syrup is sent to the burner.

Part of the evaporator condensate, along with other wastewater, is treated by anaerobic and aerobic digestion. The biogas (high in methane) from the anaerobic digestion is sent to the burner for heat recovery. The treated water is considered suitable for recycle and is returned to the process.

The solids from distillation, the concentrated syrup from the evaporator, and biogas from anaerobic digestion are combusted in a fluidized-bed combustor to raise steam for process heat. The majority of the steam demand is in the pretreatment reactor and distillation areas. Generally, the process produces excess steam that is converted to electricity for use in the plant and for sale to the grid.

6.2.6.4 Ethanol from Newspaper via Enzymatic Hydrolysis

There were such plants that were in operation in Germany (and Russia) in the 1930s. However, later on a lot of research has been carried out at the Natick Development Center (NDC) and at the University of California (Berkeley) to design and evaluate a process for the enzymatic hydrolysis of newsprint. Newspaper was chosen as the substrate because it has a high cellulose content, its composition is consistent, and it is readily available.

The process is shown in Fig. 44. A major part of the overall process cost is the production of enzymes. Another major part of the overall process cost is substrate pretreatment. Ball milling has been found to be the most effective physical method of making the maximum amount of cellulose in the substrate available for enzymatic hydrolysis, but it is expensive.

6.2.6.5 Ethanol from Municipal Solid Waste via Acid Hydrolysis

Municipal solid waste (MSW) could be also used for ethanol production. A composition of this waste is given in Tab. 21 (Ackerson, 1991). Cellulose is a valuable component of municipal primary wastewater solids and may offer an

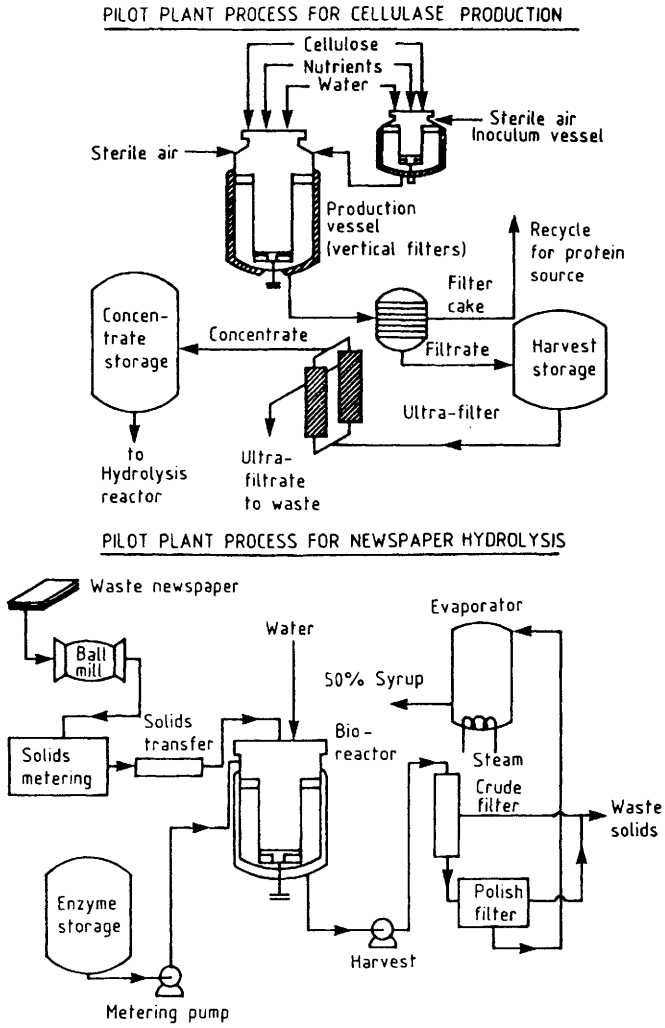

Fig. 44. Enzymatic hydrolysis of newsprint by Natick Development Center (NDC) (Spano, 1976).

alternative source for ethanol production. Estimates show that the cellulose in wastewater produced by a city of 500,000 people could be converted to just less than 4 million liters of 95% ethanol annually (Cheung and Anderson, 1996). The cellulose-containing components in this waste are essentially the raw materials for conversion to sugars by acid hydrolysis. Single- and two-step hydrolysis, with nearly 100% yields have been reported (Ackerson, 1991). The resulting sugar solution has been successfully fermented to ethanol. Best conditions for hydrolysis were obtained at high acid concentrations (80% H_2SO_4 or 41% HCl) and a temperature of 40 °C.

Tab. 21. The Composition of Selected Biomass Materials (Ackerson, 1991)

Material	Dry Weight of Material [%]		
	Hemicellulose	Cellulose	Lignin
Tanbark oak	19.6	44.8	24.8
Corn stover	28.1	36.5	10.4
Red clover hay	20.6	36.7	15.1
Bagasse	20.4	41.3	14.9
Oat hulls	20.5	33.7	13.5
Newspaper	16.0	61.0	21.0
Processed MSW	25.0	47.0	12.0

The hydrolyzates from lignocellulose contain not only readily fermentable hexoses but also a high proportion of pentoses. By utilizing also the pentose component (containing predominantly xylose from hemicellulose) a potential 30% increase in yield of ethanol can be achieved (Lawford and Rousseau, 1991). Fig. 45 represents a schematic for the production of ethanol from both cellulose and hemicellulose.

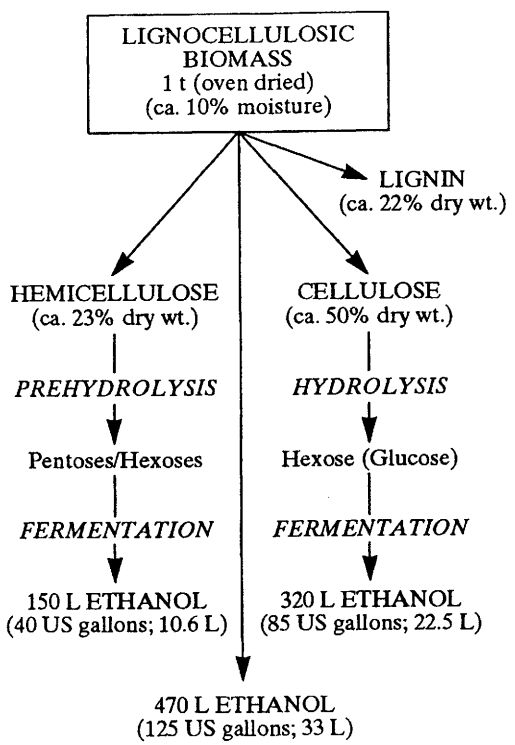

Fig. 45. Processing lignocellulosic feedstocks for the production of fuel alcohol and the theoretical maximum yield of ethanol from biomass.

In order to efficiently utilize xylose, genetically engineered *E. coli* bacteria (ATCC 11303) carrying plasmid pLOI297 with genes for pyruvate decarboxylase and alcohol dehydrogenase cloned from *Zymomonas*, were used (Lawford and Rousseau, 1991). Further work on the production of ethanol from xylose by bacteria carrying genes from *Zymomonas* is summarized in Tab. 22.

The results of the research conducted by Lawford and Rousseau are presented in Tabs. 23 and 24. Conditions were examined in pH-controlled stirred tank reactors for their effect on growth and metabolism, such as pH, xylose concentration, nutrient-rich and chemically-lean media, utilization of C_6/C_5 mixtures, sensitivity to oxygen, and tolerance of acetic acid. The recombinant converted a hardwood (aspen) hemicellulose hydrolyzate (3.5% xylose) to ethanol at an efficiency of 94%.

Gray (1999) describes the development of a planned facility in Middletown, New York, that will use municipal waste streams to make clean-burning fuel. The Orange Recycling and Ethanol Production Facility is the first commercial facility designed to recover recyclables from MSW and utilize the residuals with biosolids as feedstock for ethanol production. The facility, which is expected to open in 2001 and cost over $150 million, will have the capacity to process about 230,000 tons a^{-1} of MSW and 49,000 dry tons a^{-1} of biosolids.

Tab. 22. Production of Ethanol from Xylose by Bacteria Carrying Genes from *Zymomonas*[a] (Lawford and Rousseau, 1991)

Host Organism	Genes	Substrate	Maximal EtOH Efficiency		Investigators
			[% by wt.]	[%]	
E. coli JM 101 (pZAN4)	*pdc, adh*B	xylose	0.37	71	Neale et al. (1988)
E. coli B	"PET" plasmid	xylose	4.2	102	Ingram et al. (1989)
(pLOI 297)	*pdc, adh*B	xylose	3.9	96	Ohta et al. (1990)
E. coli S17-1	"PET" plasmid	xylose	1.0	39	Beck et al. (1990)
(pLOI 308-10)	*pdc, adh*B	glu:xyl 1:1	1.6	63	
Erwinia chrysanthemi	*pdc*	xylose	0.5	87	Tolan and Finn (1987a)
Klebsiella planticola	*pdc*	arabinose, xylose	2.5	71 78	Tolan and Finn (1987b)

[a] The majority of these fermentations were conducted anaerobically in batch mode using buffered, nutrient-rich, complex media; *pdc* is the gene for pyruvate decarboxylase; *adh*B is the gene for alcohol dehydrogenase II – both from *Zymomonas mobilis* CP4.
These genes constitute the "PET" operon ("**P**ortable **ET**hanol **P**athway; Ingram, 1990).

Tab. 23. Ethanol Production from Hardwood Hemicellulose Hydrolyzate by Recombinant *E. coli* ATCC 11303 (pLOI 297) (Lawford and Rousseau, 1991)

Conditions[a] Medium Comp. Expt.	Xylose	Products		Productivity		Yield		
		Biomass[b]	EtOH	Q_p	Q_p^{max}	q_b	$Y_{p/s}$	Conversion Efficiency
	[g L^{-1}]	[gdw L^{-1}]	[g L^{-1}]	[gP L^{-1} h^{-1}]		[gP g cell^{-1} h^{-1}]	[g g^{-1}]	[%]
Aspen prehydrolyzate + complex nutrients (LB) 13 mM Acetate (no $Ca(OH)_2$) APH3	36.1	0.5	15.5	0.29	0.34	ND	0.45	92
Aspen prehydrolyzate + complex nutrients (LB) 103 mM Acetate (+$Ca(OH)_2$[c] APH4	35.9	0.5	16.9	0.60	0.78	ND	0.47	92
Aspen prehydrolyzate + mineral salts (+$Ca(OH)_2$)[c] APH5	31.0	0.5	14.9	0.26	0.65	ND	0.48	94

[a] The pH was controlled at 7.0 with KOH; the acetate concentration was determined by HPLC analysis and did not change during the fermentation; the monomer sugars in the "prehydrolyzate" were predominantly xylose; the process yield ($Y_{p/s}$) was based on the amount of fermentable sugar in the medium; P: ethanol; ND: not determined.

[b] Approximate initial cell density (inoculum; g dry substance of cells per liter).

[c] Powdered $Ca(OH)_2$ was added to the aspen "prehydrolyzate" to pH 10 and neutralized to pH 7 with 1 N H_2SO_4 followed by centrifugation to remove insolubles.

Tab. 24. Continuous Fermentations in Ethanol Production by Recombinant *E. coli* ATCC 11303 (pLOI297) (Lawford and Rousseau, 1991)

Conditions		Substrates		Product	Productivity		Yield	
pH	Dilution Rate [h^{-1}]	Concentration [g L^{-1}]	Utilization [%]	EtOH [g L^{-1}]	q_0 [gP g cell^{-1} h^{-1}]	Q_p [gP L^{-1} h^{-1}]	$Y_{p/s}$ [g g^{-1}]	Conversion Efficiency [%]
Mineral Salts Medium								
Glucose								
6.8	0.100	31	100	9.7	1.26	0.97	0.31	61
6.3	0.081	36	94	12.3	0.72	1.00	0.36	71
6.0	0.137	31	100	14.1	0.71	1.93	0.45	88
Xylose								
6.0	0.97	33	54	7.8	0.54	0.76	0.44	86
Mixture		Glu Xyl						
6.3	0.100	21.4 12.0	100	11.2	0.65	1.12	0.34	67
6.3	0.214	21.4 12.0	76	11.4	1.26	2.44	0.45	88
6.0	0.106	22.9 12.2	96	11.4	0.86	1.21	0.34	67
6.0	0.149	22.9 12.2	81	8.8	0.62	1.31	0.31	61

6.2.7 Ethanol from Waste Sulfite Liquor (WSL)

The process of fermenting the sugars present in WSL was first investigated at a large scale in two plants in Sweden (Wallin, 1907, 1908; Ekström, 1908). In 1940, the world production was over 76.0×10^6 L with Sweden producing approximately half of the total. During the period of 1948–1950, 32 Swedish mills produced ca. 114×10^6 L a^{-1} of 95% alcohol. Today, only a few plants are left in operation in Sweden. This reduction reflects the shift from sulfite pulping to Kraft processes (Shreve and Brink, 1977), and the fierce competition with the petroleum industry for the alcohol market. During World War II, two plants were built in North America, one at Ontario Paper Co. in Thorold, Ontario, with a capacity of 7.6×10^3 L d^{-1} of 95% ethanol, and the other at Puget Sound Pulp and Timber Co. in Bellingham, Washington, with a capacity of 30.0×10^3 L d^{-1} of 95% ethanol. Since then, Commercial Alcohols Ltd. has constructed a sulfite alcohol plant at Gatineau, Quebec, which is similar in size to the Bellingham plant.

In the plant at Bellingham, the WSL from the flow tank is drained, steam stripped, and stored at about 90 °C. Fermentation is carried out continuously in 7 interconnected fermenters of 300 m^3 each. Yeast and ammonia or urea are added to the liquor entering the first fermenter. The liquor overflows from the first into the second and so on to the last fermenter. Approximately 95% of the fermentable hexose sugars are utilized in a cycle of 15–20 h. About 80% of sugars are fermented in the first two tanks and another 15% in the remaining tanks by *Saccharomyces cerevisiae*. The fermented liquor contains about 1% yeast by volume and is separated by centrifugation into two streams, one containing 15% by volume which is returned to the first fermenter and one clear liquor stream, which flows into beer storage prior to distillation. Part of the fermented liquor by-passes around the centrifuge to the beer storage. This process provides a continuous purge of dead yeast and insoluble solids that would otherwise overload the centrifuges. The clarified liquor (beer) is passed by gravity to a storage tank and distilled. Finally, the alcohol is rectified to 95 vol.%. About 95 L of fusel oil and 950 L of methanol are separated daily.

6.2.8 Ethanol from Whey

At present, the commercial fermentation of whey is done by batch as well as continuously with the recycle mode. Carbery Milk Products in Ireland produces alcohol from whey by a batchwise mode (Hansen, 1980). The whey from the cheddar cheese factory passes through a sieve after which it is separated. The whey continues on to a complex ultrafiltration section with a total capacity of 600×10^3 L d^{-1} of whey. The protein concentrate is spray-dried. The liquid permeate then continues to 6 fermentation tanks, each with a capacity of about

200 m^3. The lactose is converted to alcohol at 86% of the theoretical yield. The plant produces 14×10^3 L d^{-1} of 96.5% alcohol.

Economic analysis of alcohol production from whey has shown that it can compete with the production of synthetic alcohol from ethylene. A plant for the production of potable and industrial alcohol has operated in the United States since 1977 (Bernstein et al., 1977).

Because glucose and galactose are more universally fermentable sugars than lactose, it is suggested that β-galactosidase-treated whey would make a better substrate for industrial fermentation. This aspect has been investigated by O'Leary et al. (1977). In a subsequent study, Reese (1975) found that an ethanol yield of 6.5% could be obtained using *Saccharomyces cerevisiae* with a lactase-hydrolyzed acid whey permeate containing 30–35% total solids.

Cheese whey can also be used to replace water in the extraction of sugars from different plants, e.g., Jerusalem artichoke, for subsequent ethanol fermentation (Kosaric and Wieczorek, 1982).

7 By-Products of Ethanol Fermentation

7.1 Waste Biomass

Due to the anaerobic nature of the ethanolic fermentation, the overall synthesis of biomass is limited. In general, a 10% substrate feed with 95% conversion to alcohol will yield 5.0 g L^{-1} of dried cell mass. Therefore, separation of the fermenting organisms for cell recovery may not prove to be economically feasible.

For recycle processes, a return of 35–40% of the total biomass in the broth is all that is required to meet fermentation demands. Since the concentration step has been carried out, the remainder may be utilized in by-product markets.

After concentration, microbial biomass may be dried and utilized as a high protein food or feed supplement. Other novel uses for spent brewer's yeast have been studied by Koivurinta et al. (1980). These include their application in various food systems for the stabilization of water/oil emulsions and as a substitute for egg white because of its foaming properties.

7.2 Stillage

Stillage consists of the non-volatile fraction of material remaining after alcohol distillation. The composition of this effluent varies as a function of location and the type of feedstock used to produce it. The characteristics of stillage arising from representative crops are shown in Tab. 25. In general, these slops consist

of less than 10% solids of which 90% is protein from the feedstock and spent microbial cells. The remainder is made up of residual sugars, residual ethanol, waxes, fats, fibers, and mineral salts.

With conventional techniques, ethanol fermentation is favored by a low concentration of reactants (i.e., 12–20% of aqueous feedstock solutions). Thus, the generation of stillage may amount to 10 times the production volume of alcohol. This fact, coupled with the high BOD value of the effluent (~ 20 g L^{-1}) has led to concern over its pollution potential. Jackman (1977) estimated that in a traditional cane molasses distillery (100,000 L d^{-1} anhydrous ethanol) the resulting pollution load would be equivalent to a city population of 1.7×10^6 persons. Therefore, any processes which would utilize this effluent as by-product would also generate credit as a means of pollution control.

Recovery processes for stillage are numerous. Fig. 46 illustrates some of the main process routes and the possible applications of resulting products. In Brazil, some stillage has been returned to the cane fields as fertilizer and for irrigation (Castello-Branco et al., 1980). This may be a desirable alternative to disposal since the high potash and organic matter of the effluent can be beneficial to a certain level in crop production. Problems encountered by this application involve increases in solid acidity and salt concentration as well as the extremely pungent odor of the putrefying liquid.

Tab. 25. Mean Composition of In-Nature Stillages Produced in Brazilian Ethyl Alcohol Distilleries (Costa-Ribeiro and Castello-Branco, 1981)

Parameter	Type of Stillage			
	Molasses [g L^{-1}]	Cane-Juice [g L^{-1}]	Mixed [g L^{-1}]	Mandioca [g L^{-1}]
Total solids	81.5	23.7	52.7	22.5
Volatile solids	60.0	20.0	40.0	20.0
Fixed solids	21.5	3.7	12.7	2.5
Carbon (as C)[a]	18.2	6.1	12.1	6.1
Reducing substances	9.5	7.9	8.3	6.8
Crude protein[b]	7.5	1.9	4.4	2.5
Potassium (as K_2O)	7.8	1.2	4.6	1.1
Sulfur (as SO_4)	6.4	0.6	3.7	0.1
Calcium (as CaO)	3.6	0.7	1.7	0.1
Chlorine (as NaCl)	3.0	1.0	2.0	0.1
Nitrogen (as N)	1.2	0.3	0.7	0.4
Magnesium (as MgO)	1.0	0.2	0.7	0.1
Phosphorus (as P_2O_5)	0.2	0.01	0.1	0.2
BOD	25.0	16.4	19.8	18.9
COD	65.0	33.0	45.0	23.4
Acidity[c]	4.5	4.5	4.5	4.5

[a] Carbon content = organic solids content: 3.3
[b] Crude protein content = nitrogen content × 6.25
[c] Expressed in pH units

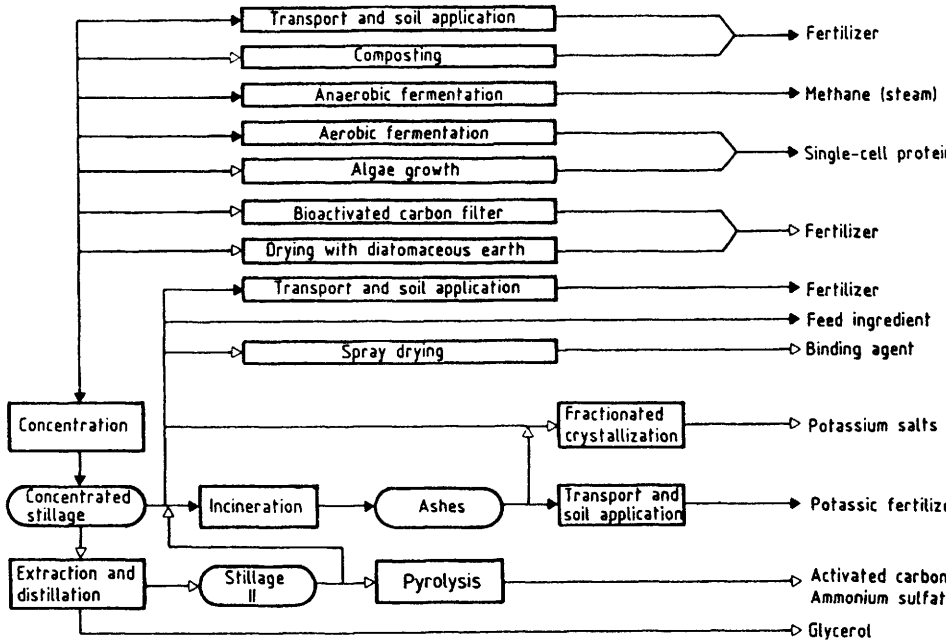

Fig. 46. Stillage recovery processes (Costa-Ribeiro and Castello-Branco, 1981). ——▸ main products; ——▷ other products.

Wet stillage has a high protein content and as such can be fed directly to local feedstock in the form of a warm slurry. Since the overall nutrient composition of this material is limited, it would require fortification with other feed sources to ensure a balanced diet (Wyvill and Battaglia, 1981). Evaporation of the effluent to a thick syrup or powder would extend its storage capacity. Care must be taken to ensure that salt concentrations do not exceed a level detrimental to livestock health.

If evaporation is used to concentrate the stillage, the resulting water or condensate may be recycled to the process stream. This would result in water savings and a decrease in effluent volumes. The use of raw stillage for re-extraction of the feedstock is feasible. However, the build-up of inhibition products and the increase in osmotic pressure due to non-fermentable products would need to be carefully controlled.

Operations in Taiwan have utilized new stillage as feedstock in the production of Torula yeast (*Candida utilis*) for almost a decade (Chang and Yang, 1973). Interest has been shown in the use of other fungi for this type of process (Gonzales and Murphy, 1980).

Fig. 47 illustrates an integrated system developed by Sang et al. (1980) which involves continuous yeast growth on raw alcohol slops and subsequent culture of mold biomass on yeast wastes. These researchers were able to reduce the

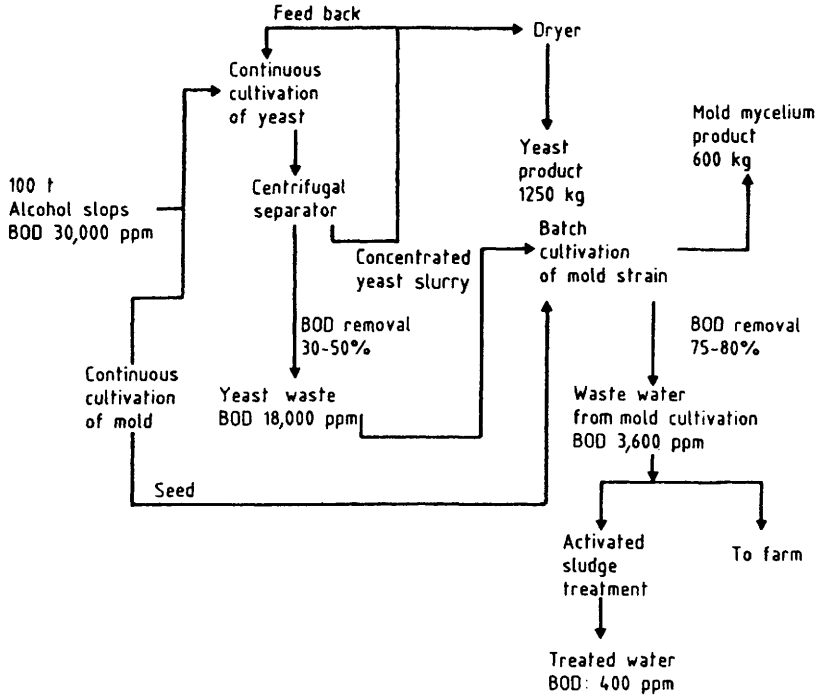

Fig. 47. Flow diagram of a proposed process for complete treatment of alcohol slops (Sang et al., 1980).

overall BOD of stillage by 90% with a simultaneous yeast productivity of 4.2 g L^{-1} h^{-1}.

Factors which will greatly affect the potential of microbial protein production from stillage include the need for exogenous nutrient fortification (i.e., N and P) at additional costs, high initial capital required for plant construction, and a high energy demand for unit processes (especially fermenter cooling in tropical countries).

Waste effluents from distillery operations are also amenable to anaerobic digestion. Operating at mesophilic temperatures (32 °C) and adequate hold-up times, 95% of the original BOD value can be eliminated. Sludge gas with a 65% (v/v) methane content may be produced at a yield of 580–720 L kg^{-1} BOD removed. The net heat value of this product is ca. 25 MJ m^{-3} and as such may provide boiler steam to drive the unit processes of the distillery (Jackman, 1977).

An additional credit is that nitrogen remains in the effluent of the digester as ammonium salts and as such is a good source of organic nitrogen for crop fertilization. The increased pH of digested stillage (final pH 7.4) also makes it more suitable for direct land application (Sanchez-Riera et al., 1982).

7.3 Carbon Dioxide

For every m^3 of ethanol formed, about 760 kg of CO_2 are liberated from the fermentation broth. Of this total, 70–80% can be recovered in a closed system. After purification to remove aldehydes and alcohols, the gas may be stored in cylinders or further compressed to solid or liquid form.

The market value of CO_2 is relatively variable. It is not economical to transport it a great distance from the plant. In the gaseous state, it may be used to carbonate soda beverages or to enhance the agricultural productivity of greenhouse plants. Liquid CO_2 is frequently used in fire extinguishers, refrigeration processes, and as a feedstock in the chemical industry. The primary use of solid CO_2 is as a refrigerant.

7.4 Fusel Oils

Fusel oils are formed from α-keto acids, derived from or leading to amino acids. The overall composition is found to be an isomeric mixture of primary methyl butanols and methyl propanols, the majority of which is isoamyl alcohol (Maiorella et al. et al., 1981). Tab. 26 compares the composition of fusel oils from different sources. Yields of up to 20 L may be attained per m^3 of ethanol generated using standard sugar sources, however, this value depends upon the pH in the fermenter.

Fusel oils are found to come of the distillation tower at relatively high temperatures and must be completely removed, or plugging of the column may result (Combes, 1981). Due to its similarity to the major components in gasoline, the use of this by-product would be as a further fuel extender or as an industrial solvent.

Tab. 26. Average Composition of Various Fusel Oils [vol.%] (Pfenninger, 1963)

Component	Molasses Fusel Oil	Cereal Fusel Oil	Potato Fusel Oil	Fruit Fusel Oil	Baker's Yeast Fusel Oil	Sulfite Liquor Fusel Oil
n-Propylalcohol	8.6	9.1	16.4	10.2	11.0	6.8
sec-Butylalcohol	–	–	–	2.4	6.2	–
Isobutylalcohol	20.6	19.2	15.9	21.4	23.8	22.3
n-Butylalcohol	0.5	0.2	1.2	2.2	2.3	0.7
Opt. act. amylalcohol	31.3	19.0	13.6	13.6	19.0	13.1
Isoamylalcohol	39.1	52.4	52.9	56.4	37.7	56.1
n-Amylalcohol	–	–	–	–	–	1.0

8 Economic and Energy Aspects of Ethanol Fermentation

A number of economic analyses related to large-scale production of ethanol are available (Scheller and Mohr, 1976; Scheller, 1976; De Carvalho et al., 1977; Lipinsky et al., 1977; Intergroup Consulting Economists Ltd., 1978; Fayed et al., 1981; Misselhorn, 1980; Novo Industri, 1979; Slesser and Lewis, 1979; Hofreither et al., 1987; Ackerson, 1991; Mistry, 1991; Bridgewater and Double, 1991, 1994; Marsh and Cundiff, 1991; Dunne, 1994; Woodley et al., 1999a,b). The estimated figures as presented vary and rapidly become outdated as inflation increases energy costs, capital costs, operating expenses, and crop prices.

A distribution of costs for ethanol production from various raw materials is shown in Fig. 48. According to Mistry (1991) feed and processing costs vary depending on the raw material (Tab. 27). For different processes, the costs are broken down as shown in Fig. 49. The characteristics of the above processes are presented in Tab. 28. Capital and operating costs for plants operating at different processes are presented in Tabs. 29 and 30. The production costs and cost contributions based on data evaluated for the 5 different processes presented above, are shown in Tabs. 31 and 32. The cost contributions, except that of feed, are shown in Fig. 50.

A cost analysis for conversion of sweet sorghum to ethanol was presented by Marsh and Cundiff (1991) for a U.S. scenario (Piedmont and Louisiana). Cost to provide year-round, readily fermentable, sweet sorghum feedstock to a central plant in the Piedmont was determined to vary from \$0.52–0.79 per liter (\$1.96–2.98 per gallon) ethanol potential. Cost to provide feedstock employing the established sugarcane-to-sugar industry in Louisiana is shown to be \$0.66 per liter (\$2.50 per gallon). Assuming cellulose feedstock is valued at \$42.00 t^{-1} dry matter delivered to the ethanol production facility, sweet sor-

Tab. 27. Distribution of Ethanol Production Cost (Mistry, 1991)

Feed	Feed Cost Contribution [%]	Processing Cost Contribution [%]
Sugarcane	50–83	17–50
Molasses	78–83	17–22
Sugar beet	50–68	32–50
Cassava	60–75	25–40
Corn	53–87	13–47
Wheat	40–75	25–60
Maize	60–70	30–40
Wood wastes	9–42	58–91
Algae	62	38
Wheat straw	9–23	77–91

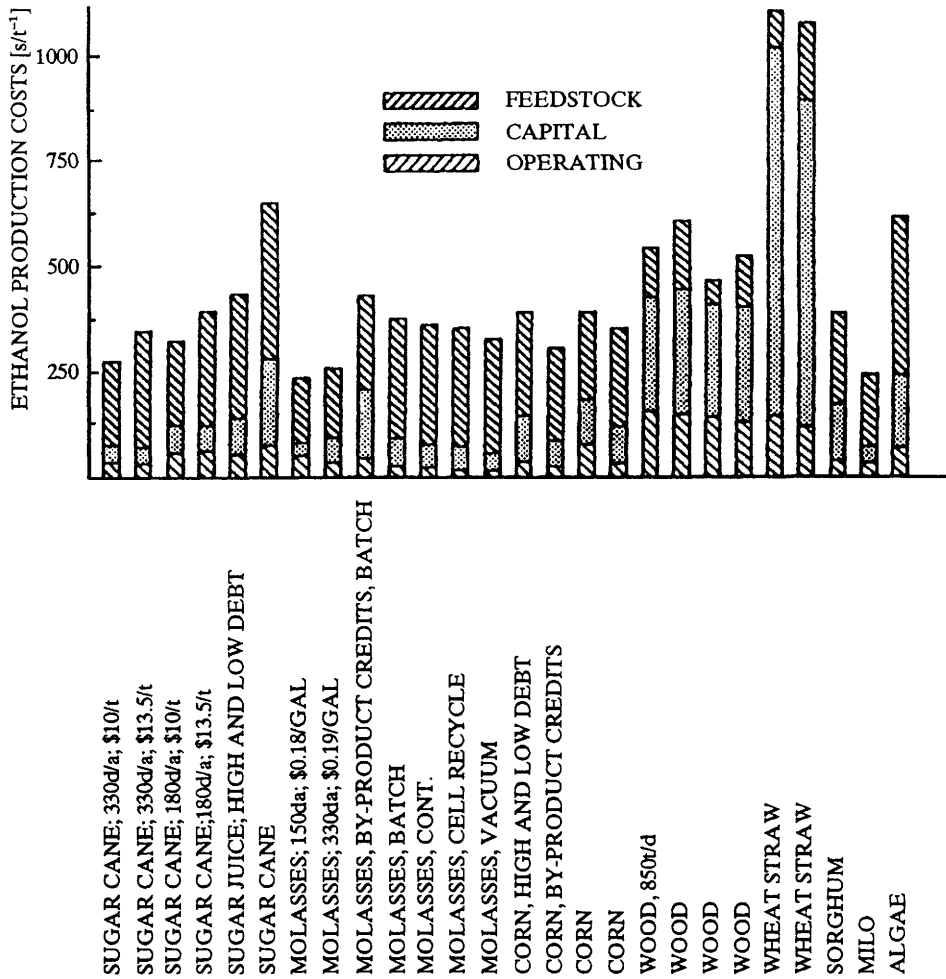

Fig. 48. Distribution of ethanol production costs.

ghum by-products are reported to have more value as cattle feed (Marsh and Cundiff, 1991).

According to Dunne (1994), the energy inputs and economics from conventional agricultural crops are shown in Tab. 33. The estimated ethanol conversion costs for selected plant sizes are shown in Tab. 34. The estimated feedstock and conversion costs for selected crops are shown in Tab. 35.

Woodley et al. (1999b) indicate that savings resulting from economies of scale are offset by increased costs for feedstock collection. Put quite simply, the more feedstock a plant demands, the farther out it must go to get it. Collection distance for a plant is highly site specific.

Tab. 28. Technical Parameters Used in Different Processes for Ethanol Production (Mistry, 1991)

Flowsheet	1 Continuous Yeast Process	2 Continuous Thermophilic Process	3 Vacuum Thermophilic Process	4 CO_2 Recycle Thermophilic Process	5 Current Practice Yeast Process
Sucrose concentration to sterilizer [wt.%]	10.0	10.0	16.6	16.6	16.8
Sucrose concentration to anaerobic stage [wt.%]	7.7	8.7	15.0	15.0	(16.8)[a]
Temperature [°C]	35	70	70	70	35
Biomass yield [kg kg S^{-1}]	0.128	0.59	0.039	0.039	0.108
Ethanol yield [kg kg S^{-1}]	0.409	0.435	0.454	0.454	0.433
Specific productivity, q_p [kg kg biomass h^{-1}]	0.63	1.3	1.3	1.3	0.40
Volumetric productivity, r_p [kg m^{-3} h^{-1}]	31.5	31.5	31.5	31.5	31.5
Biomass concentration [kg dry mass m^{-3}]	50	24.2	24.2	24.2	78.2
Biomass recycle [%]	75	75	75	75	75
Ethanol conc. in beer, P_w [wt.%]	4.0	4.0	4.0	4.0	7.8
Sucrose conversion [%]	98	98	98	98	98

[a] Aerobic and anaerobic stages are not separate for process 5.

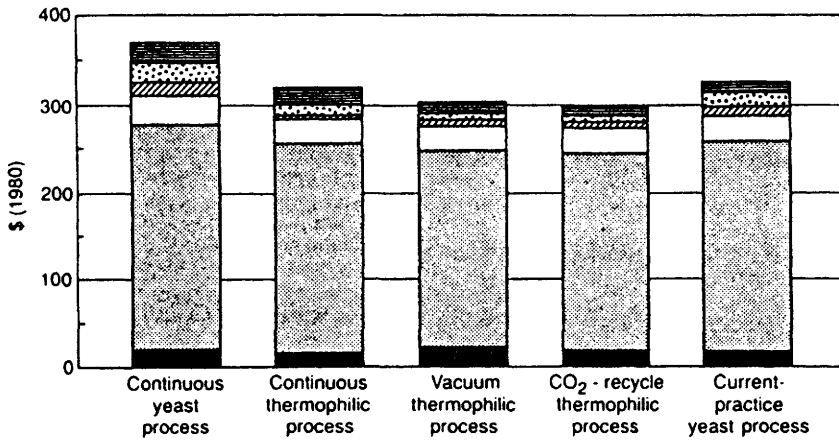

Fig. 49. Histograms showing cost contributions in producing 1 m^3 ethanol; ■ fixed charges, ⊞ feed, □ steam, ▨ water, ⊡ chemicals, ▤ stillage treatment.

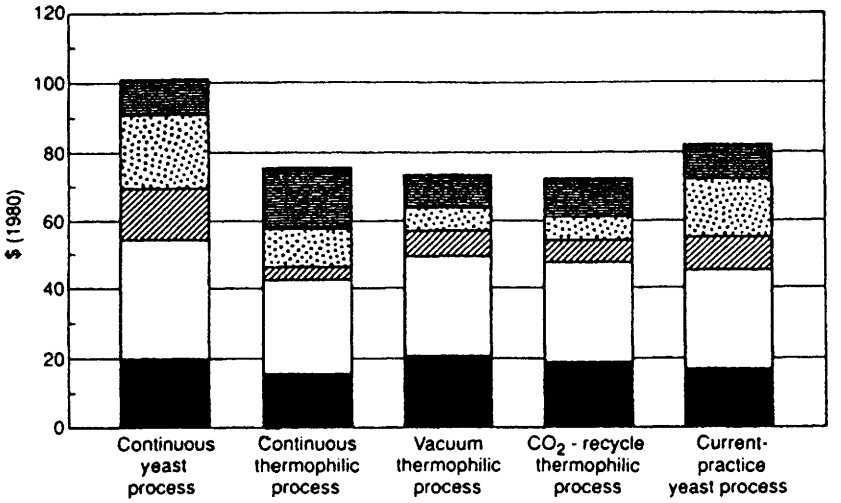

Fig. 50. Histograms showing cost contributions, except that of feed, in producing 1 m^3 ethanol; ■ fixed charges, □ steam, ▨ water, ▦ chemicals, ▤ stillage treatment.

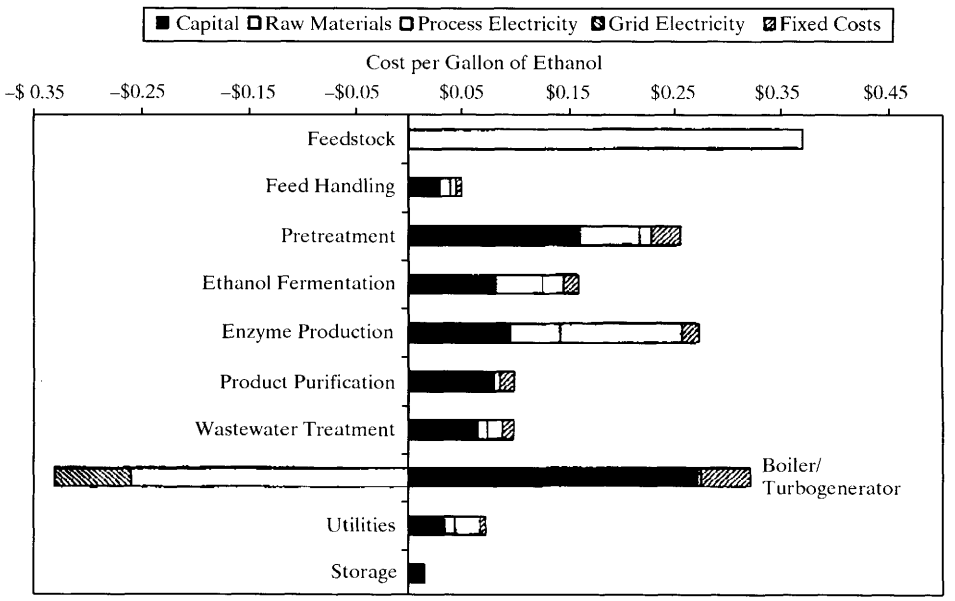

Fig. 51. Cost contribution details of the different units in an ethanol production plant (Woodley et al., 1999b).

Collecting biomass for the plant has two main costs:

(1) The cost of harvesting and baling.
(2) The cost of transportation from the farm to the plant gate.

Tab. 29. Components of Capital Costs of Ethanol Production in 1980 (Mistry, 1991)

Flowsheet	1 Continuous Yeast Process [$]	2 Continuous Thermophilic Process [$]	3 Vacuum Thermophilic Process [$]	4 CO_2 Recycle Thermophilic Process [$]	5 Current Practice Yeast Processes [$]
Evaporator	–	–	0.270	0.270	0.300
Sterilizer	0.130	0.092	0.058	0.058	0.083
Aerobic fermenter	0.101	0.065	0.035	0.035	0.133
Anaerobic fermenter	0.134	0.134	0.133	0.133	–
Fermenter cooler	0.051	0.020	–	–	0.058
Fermenter reboiler	–	–	0.015	0.014	–
Beer storage vessel	0.054	0.054	0.053	0.053	0.053
Centrifuge(s)	0.564	0.346	0.184	0.186	0.279
Compressor[a]	–	–	0.350	0.180	–
Cooler/condenser	–	0.032	0.069	0.072	–
Absorption column	0.018	0.023	0.024	0.035	0.018
Distillation column	0.123	0.217	0.100	0.105	0.069
Distillation reboiler	0.061	0.051	0.048	0.049	0.051
Distillation conditioner	0.117	0.117	0.117	0.117	0.112
Total equipment cost	1.352	1.060	1.455	1.306	1.156
Total plant cost[b]	5.408	4.240	5.820	5.224	4.624

[a] For vacuum thermophilic process: cost = cost of vapor compressor + cost of vacuum pump.
[b] Total plant cost = 4 × total equipment cost.

The type of feedstock will have the biggest effect on the feedstock-handling portion of the process. Additionally, the feedstock composition certainly will have an impact on pretreatment yields and on how much ethanol is produced, as well as an effect on the efficiency of the fermenting organism.

Plants that are being engineered today all have some niche that allows them a special advantage in the short term for a small market segment. This could be feedstock costs (very low or negative for environmental wastes), used equipment (making use of related equipment that has been shut down), co-location with existing facilities (biomass burners and waste treatment facilities), or a combination of these. Cost contribution details of each step in an ethanol production plant is given in Fig. 51.

Tab. 30. Production Costs in 1980 (Mistry, 1991)

Flowsheet	1 Continuous Yeast Process [$]	2 Continuous Thermophilic Process [$]	3 Vacuum Thermophilic Process [$]	4 CO_2 Recycle Thermophilic Process [$]	5 Current Practice Yeast Processes [$]
Plant cost [M$]	5.409	4.420	5.810	5.224	4.624
Annual running cost [M$]	14.637	12.628	11.658	11.745	12.822
Production cost[a, b] [M$ m^{-3}]	368	316	298	298	322

[a] Production cost is evaluated by taking 15% of total plant cost as the fixed capital *per annum*, and for a 350 day year.
[b] Cost per m^3 anhydrous ethanol ≙ cost per 1.05 m^3 of 93.5 wt.% ethanol.

Tab. 31. Annual Running Costs (1980) for Flowsheets 1–5, Based on Production of 120 m^3 d^{-1} Anhydrous Ethanol (Mistry, 1991)

Flowsheet	1 Continuous Yeast Process [$]	2 Continuous Thermophilic Process [$]	3 Vacuum Thermophilic Process [$]	4 CO_2 Recycle Thermophilic Process [$]	5 Current Practice Yeast Processes [$]
Cane[a]	10.820	10.100	9.450	9.494	10.085
Chemicals[b]	0.902	0.450	0.290	0.292	0.727
Steam[c]	1.423	1.158	1.191	1.207	1.197
Water[d]	0.639	0.148	0.310	0.277	0.406
Stillage treatment[e]	0.853	0.762	0.417	0.475	12.822
Total	14.637	12.628	11.658	11.745	12.822

[a] 1,200 kg of 20 wt.% sucrose solution costs 15 $ (1980).
[b] Chemical cost = 0.075 kg^{-1} biomass + 3 S m^{-3} anhydrous production capacity.
[c] Steam cost = 0.0035 $ kg^{-1} HP steam, based on the use sugarcane bagasse.
[d] Water cost = 0.04 $ m^{-3}.
[e] Stillage treatment cost = 1 $ m^{-3}.

An economic analysis for production of ethanol from municipal solid waste was presented by Ackerson (1991). A facility has been designed to produce 75.7×10^6 L (20×10^6 gallons) of ethanol per year, utilizing a strong acid for hydrolysis in a process prior to fermentation by *Saccharomyces cerevisiae*.

In this process, municipal solid waste (MSW, with a composition as shown in Tab. 36) was collected and delivered to the plant size as needed. The feedstock was prepared by removing plastic, metal, and glass, followed by shredding and grinding. The cost for glass and metal removal is not included. Including all the other costs (acid hydrolysis, ethanol fermentation and distillation) the total

Tab. 32. Cost Contributions (1980) in Producing 1 m^3 Ethanol (Mistry, 1991)

Flowsheet	1 Continuous Yeast Process [$]	2 Continuous Thermophilic Process [$]	3 Vacuum Thermophilic Process [$]	4 CO_2 Recycle Thermophilic Process [$]	5 Current Practice Yeast Processes [$]
Fixed charges	19	15	21	19	17
Feed	258	241	225	226	240
Steam	35	20	28	29	29
Water	15	4	7	7	10
Chemicals	22	11	7	7	17
Stillage treatment	20	18	10	11	10
Total	368	316	298	298	322

Tab. 33. Energy and Financial Inputs Required to Produce Liquid Fuels[a] from Conventional Agriculture Crops (Dunne, 1994)

Crop	Energy Input [MJ L^{-1}]	Feedstock Cost	By-Product Value	Feedstock Cost less By-Product Value
Winter wheat	8.75	22.8	11.0	11.8
Spring wheat	8.39	24.1	11.0	13.1
Winter barley	10.09	28.1	14.9	13.2
Spring barley	8.21	23.0	14.9	8.1
Winter oats	12.53	32.0	24.7	7.3
Spring oats	12.49	38.9	24.7	14.2
Sugar beet[b]	4.64	19.0	7.5	11.5
Sugar beet[c]	3.93	16.1	9.3	6.7
Fodder beet[b]	4.96	22.1	9.4	12.7
Fodder beet[c]	3.97	17.6	11.3	6.3
Potatoes	16.99	85.1	11.1	74.0
Swedes	13.18	51.5	17.5	34.0
Grass 1 cut	17.36	37.8	80.0	−42.2
Grass 2 cuts	25.80	51.1	80.00	−28.9
Grass 3 cuts	30.87	59.5	80.00	−20.5
Grass 4 cuts	39.10	71.3	80.00	− 8.7
Oilseed rape	19.03	41.7	10.6	31.1

[a] Liquid fuel is ethanol for all crops except oilseed rape. For oilseed rape the liquid fuel is vegetable oil.
[b] Roots only.
[c] Roots and tops.

capital cost is reported to be $35 million including all utilities, storage, and offsites.

The annual operating costs are shown in Tab. 37. A lignin boiler is used to reduce the energy requirements so that energy costs are reduced to only $0.02 per liter ($0.08 per gallon). Fixed charges are computed as a percentage of the

Tab. 34. Estimated Ethanol Conversion Cost for Selected Plant Sizes (Dunne, 1994)

Plant Size [d^{-1}]	Capital Costs [IR pence L^{-1}]	Other Costs [IR pence L^{-1}]	Total Costs [IR pence L^{-1}]
25,000	29.1	18.2	47.3
50,000	21.8	15.9	37.7
100,000	16.5	15.3	31.8
200,000	12.6	14.1	26.7

Tab. 35. Estimated Feedstock and Conversion Costs for Selected Crops (Dunne, 1994)

Feedstock	Feedstock Cost [IR pence L^{-1}][a]	Feedstock Cost less By-Product Credit [IR pence L^{-1}][a]
Winter wheat	22.8	11.8
Spring barley	23.0	8.1
Sugar beet (roots only)	19.0	11.5
Sugar beet (roots and tops)	16.1	6.7
	Cost of Feedstock Plus Conversion[b]	
Winter wheat	49.5–70.1	38.5–59.1
Spring barley	49.7–70.3	34.8–55.4
Sugar beet (roots only)	45.7–66.3	38.2–58.8
Sugar beet (roots plus tops)	42.8–63.4	33.4–54.0

[a] Feedstock cost only as per Tab. 33.
[b] Feedstock cost as per Tab. 33 plus the processing costs for both the largest and smallest plants as per Tab. 34.

Tab. 36. Municipal Solid Waste Composition (wt.% as discarded) (Ackerson, 1991)

Category	Summer	Fall	Winter	Spring	Average
Paper	31.0	38.9	42.2	36.5	37.4
Yard waste	27.1	6.2	0.4	14.4	13.9
Glass	17.7	22.7	24.1	20.8	20.0
Metal	7.5	9.6	10.2	8.8	9.8
Wood	7.0	9.1	9.7	8.2	8.4
Textiles	2.6	3.4	3.6	3.1	3.1
Leather and rubber	1.8	2.5	2.7	2.2	2.2
Plastics	1.1	1.4	1.5	1.2	1.2
Miscellaneous	3.1	4.0	4.2	3.7	3.4

capital investment and in total $5.6 million per year. At an ethanol price of ca. $0.40 per liter ($1.50 per gallon), revenues are generated in the amount of $30 million, yielding a pre-tax profit of $18.5 million per year (ca. $0.25 per liter, resp. $0.93 per gallon) or 53% per year. However, utilization of pentoses is not considered in this analysis. Acid recovery is included, but fermentation of xylose is not provided. According to other data (Lawford and Rousseau, 1991), a

Tab. 37. Economics of a $75.6 \cdot 10^6$ L a^{-1} Ethanol Facility[a] (Ackerson, 1991)

Capital cost:

	Million $
Feedstock preparation	3.0
Hydrolysis	5.0
Acid recovery	8.5
Fermentation and purification	8.0
Utilities/offsites	6.5
Engineering	4.0
	35.0

Operating cost:

	Million $ per year	$ per liter (per gallon)
MSW	–	– –
Utilities	1.5	0.021 (0.08)
Chemicals	1.9	0.034 (0.09)
Labor	2.5	0.034 (0.13)
Fixed Charges		
Maintenance (4%)	1.4	0.018 (0.07)
Depreciation (10%)	3.5	0.048 (0.18)
Taxes and insurance (2%)	0.7	0.005 (0.02)
Pre-tax profit (53%)	18.5	0.246 (0.93)
	30.0	0.396 (1.50)

[a] $20 \cdot 10^6$ gallons per year.

Tab. 38. Cost Comparison for Production of Ethanol (100 vol.%) for Different Plant Sizes (Kosaric et al., 1982b)

Costs	Plant Size		
	$3 \cdot 10^5$ kg	$3.6 \cdot 10^5$ kg	$4 \cdot 10^6$ kg
Fixed operating costs (depreciation[a], maintenance and repairs, labor, taxes, interest)	0.75	0.63	0.30
Direct operating costs (supplies, steam, power, water)	0.20	0.20	0.29
Raw material cost[b] (Jerusalem artichokes)	0.17	0.17	0.17
Cost of 1 kg ethanol	1.12	1.00	0.76
By-product credits			
Pulp (17% protein d.m.)	0.39	0.39	0.39
Stillage (protein 18.5 kg 1,000 L^{-1})	0.16	0.16	0.16
Net cost of ethanol $ L^{-1}	0.55	0.43	0.21

[a] Buildings at 20 years amortization, equipment at 100 years amortization.
[b] Taken at U.S. $ 10 per ton.

30% increase in alcohol yield would be expected if xylose was also fermented to ethanol. One could estimate, therefore, that considerably better economics would be achieved by utilization of the pentoses (available in the acid hydrolyzate), for ethanol production.

Kosaric et al. (1982) have made an economic evaluation for the small-scale production of ethanol (farm size) for Jerusalem artichoke as raw material. Tab. 38 shows the costs of ethanol production for different plant sizes. The cost of ethanol produced in the plant with a capacity of 4×10^6 kg a^{-1} was estimated to be about 40% of the price of gasoline in Canada in 1995.

8.1 Ethanol from Jerusalem Artichokes (A Case Study)

The cost for producing Jerusalem artichokes at the farm level and the cost of processing the Jerusalem artichokes (tops and tubers) to ethanol have been in-

Tab. 39. Annual Cost of Production of Tubers (Quebec)

Land cost per ha	Costs [$]			
	2,500	1,750	1,100	675
Total variable cost	1,719	1,719	1,719	1,719
Total fixed cost	1,029	906	800	731
Storage and transportation	1,052	1,052	1,052	1,052
Total cost per ha	3,800	3,677	3,571	3,502
Cost per ton (fresh matter)	92.68	89.68	87.10	85.41
Tons per ha (fresh matter)	41	41	41	41
Land cost per ha (Western Canada)			1,100	500
Cost per ton (fresh matter)			73.19	71.81
Tons per ha (fresh matter)			53	53

Tab. 40. Annual Cost of Production of Tops in Large Bales (Quebec)

Land cost per ha	Costs [$]			
	2,500	1,750	1,100	675
Total variable cost	503	503	503	503
Total fixed cost	792	669	563	494
Storage and transportation	198	198	198	198
Total cost per ha	1,493	1,370	1,264	1,195
Cost per ton (fresh matter)	46.41	33.41	30.83	29.15
Tons per ha (fresh matter)	41	41	41	41
Land cost per ha (Western Canada)			1,100	500
Cost per ton (fresh matter)			15.78	14.82
Tons per ha (fresh matter)			100	100

vestigated by Baker et al. (1990, 1991). In this study, various ethanol yields from Jerusalem artichoke feedstock and conversion efficiencies have been considered for implications of different processing plant sizes. Scenarios for Quebec and Western Canada have been presented.

The cost of production varies by system of production (i.e., tubers or tops), by land price, and by region (Tabs. 39 and 40). The total cost per ha for tuber production varied from a high of $4,432 in Eastern Canada to a low of $3,502 in Quebec. As would be expected, the cost of production decreases as the land value decreases. The lowest total cost per ton of tubers on a fresh matter basis was $71.81 in Western Canada. The advantage that Western Canada has over Quebec in tuber production is in terms of the yield (43 t ha^{-1} vs. 41 t ha^{-1}).

The total cost of production for tops in large bales per ha varied from a high of $1,493 to a low of $1,195 in Quebec on the least expensive land. Translating these costs per ha into costs per ton on a fresh matter basis, the lowest cost per ton can be found in Western Canada ($14.82). The yield advantage for tops in Western Canada, 100 t ha^{-1}, as compared to Quebec, 41 t ha^{-1}, plays an important part in providing such a low cost per ton.

The supply price which has been estimated includes a return to the operator. The return to the operator is based on a return of 3% of the value of land and buildings used to produce either the tubers or the tops from Jerusalem artichoke. The returns for tubers are higher than for tops because of the greater amount of machinery required for this type of production. The returns varying by region and type of production are given in Tab. 41.

The farm-level costs of production can be presented in terms of $ per liter of ethanol by taking into account a range of possible conversion factors, and assuming possible yields. These costs for Quebec and Western Canada for tubers are given in Tabs. 42 and 43, respectively.

Tab. 41. The Operator's Return for Jerusalem Artichoke

Region	Return [$ ha^{-1}]			
Quebec				
Land value	2,500	1,750	1,100	675
Tubers	149.05	126.55	107.05	94.30
Tops (large)	120.68	98.18	78.68	65.93
Tops (small)	123.25	100.76	81.26	68.51
Western Canada				
Land values			1,100	500
Tubers			160.38	142.38
Tops (large)			110.18	92.18
Eastern Canada				
Land values		1,900	1,100	
Tubers		392.04	368.04	
Tops (large)		282.78	258.78	
Tops (small)		294.08	270.08	

Tab. 42. Ethanol Feedstock Cost of Production Using Jerusalem Artichoke Tubers

Land Price [$ ha^{-1}]	2,500					675				
Yield [ha^{-1})	30.00	41.00	50.00	60.00	76.00	30.00	41.00	50.00	60.00	76.00
Conversion Factor [L t^{-1}]	Quebec [$ L^{-1}]					Quebec [$ L^{-1}]				
80.00	1.58	1.16	0.95	0.79	0.62	1.46	1.07	0.88	0.73	0.58
100.00	1.27	0.93	0.76	0.63	0.50	1.17	0.85	0.70	0.58	0.46
120.00	1.06	0.77	0.63	0.53	0.42	0.97	0.71	0.58	0.49	0.38
140.00	0.90	0.66	0.54	0.45	0.36	0.83	0.61	0.50	0.42	0.33
150.00	0.84	0.62	0.51	0.42	0.33	0.78	0.57	0.47	0.39	0.31
Land Price [$ ha^{-1}]	1,100					500				
Yield [ha^{-1}]	30.00	41.00	53.00	60.00	76.00	30.00	41.00	53.00	60.00	76.00
Conversion	Western Canada [$ L^{-1}]					Western Canada [$ L^{-1}]				
80.00	1.62	1.18	0.91	0.81	0.64	1.59	1.16	0.90	0.79	0.63
100.00	1.29	0.95	0.73	0.65	0.51	1.27	0.93	0.72	0.63	0.50
120.00	1.08	0.79	0.61	0.54	0.43	1.06	0.77	0.60	0.53	0.42
140.00	0.92	0.68	0.52	0.46	0.36	0.91	0.66	0.51	0.45	0.36
150.00	0.86	0.63	0.49	0.43	0.34	0.85	0.62	0.48	0.42	0.33

Tab. 43. Ethanol Feedstock Cost of Production Using Jerusalem Artichoke Tops

Land Price [$ ha^{-1}]	2,500					675				
Yield [t ha^{-1}]	30.00	41.00	55.00	80.00	100.00	30.00	41.00	55.00	80.00	100.00
Conversion Factor [L t^{-1}]	Quebec [$ L^{-1}]					Quebec [$ L^{-1}]				
90.00	0.55	0.40	0.30	0.21	0.17	0.44	0.32	0.24	0.17	0.13
100.00	0.50	0.36	0.27	0.19	0.15	0.40	0.29	0.22	0.15	0.12
110.00	0.45	0.33	0.25	0.17	0.14	0.36	0.26	0.20	0.14	0.11
Land Price [$ ha^{-1}]	1,100					500				
Yield [t ha^{-1}]	60.00	80.00	100.00	120.00	130.00	30.00	41.00	53.00	60.00	76.00
Conversion Factor [L t^{-1}]	Western Canada [$ L^{-1}]					Western Canada [$ L^{-1}]				
90.00	0.29	0.22	0.18	0.15	0.13	0.27	0.21	0.16	0.14	0.13
100.00	0.26	0.20	0.16	0.13	0.12	0.25	0.19	0.15	0.12	0.11
110.00	0.24	0.18	0.14	0.12	0.11	0.27	0.17	0.13	0.11	0.10

Tab. 44. Feedstock Costs for an Ethanol Industry Using Alternative Biomass Sources

Source	1989 Costs[a] [$ t^{-1}]	Conversion Factor [L t^{-1}]	Feedstock Costs [$ L^{-1}]
Corn	152.65	386.9	0.39
Barley	131.45	347.2	0.38
Wheat (feed)	130.45	347.2	0.38
Wheat (CWRS1)	247.49	347.2	0.71

[a] Feedstock prices were obtained from Agriculture Canada, Policy Branch, Ottawa (1990).

Tab. 45. Feedstock Requirements and Output for a 100×10^6 L Plant

Inputs/Outputs	Mass [t]	Value [10^6 $]
Inputs:		
Jerusalem Artichoke Tops (DMB)	293,571	
Fresh weight basis, moisture 70%	978,571	14.97
Cost (FWB) at 15.30 [$ t^{-1}]		
Outputs:		
Ethanol (anhydrous)	79,000	
Feed by-products (DMB)	139,422	
48% Soy meal price at 240.00 [$ t^{-1}]		
14% Moisture; protein 20.64%	162,119	
By-product value at 103.22 [$ t^{-1}]		16.73
CO_2 at 150.00 [$ t^{-1}]	68,256	10.24
Inputs:		
Jerusalem Artichoke Tubers (DMB)	211,513	
Fresh weight basis, moisture 80%	1,057,564	
Cost (FWB) at 72.50 [$ t^{-1}]		76.67
Outputs:		
Ethanol (anhydrous)	79,000	
Feed by-products (DMB)	58,581	
48% Soy meal price at 240.00 [$ t^{-1}]		
14% Moisture; protein 31.21%	68,118	
By-product value at 156.03 [$ t^{-1}]		10.63
CO_2 at 150.00 [$ t^{-1}]	68,256	10.24

Assuming a value of land in Quebec of $2,500 per ha, the feedstock cost per liter ethanol from tubers ranges from a high of $1.58 to a low of $0.33 and with a land value of $675 per ha the range is $1.45 to $0.31. In Western Canada the cost per liter ethanol from tubers ranged from $1.62 on the highest valued land to $0.34 and from $1.59 to $0.33 on the cheaper land. In the case of ethanol production from tops, the range of costs per liter in Quebec are $0.55 to $0.14

on the most expensive land and $0.44 to $0.11 on the lowest valued land. In Western Canada, producing tops on the best land leads to a cost range for ethanol production of $0.29 to $0.11 per liter, and on the lower quality land the range is $0.27 to $0.10.

These feedstock costs could be compared with other sources presented in Tab. 44. The feedstock requirements and output for a plant with a capacity of 100×10^6 L are presented in Tab. 45. A sensitivity analysis of the results was also done showing that the final cost for ethanol production changes as each of the number of variables is changed independently.

The ethanol cost is quite sensitive to both the feedstock cost and the value of soymeal upon which the by-product credit is based. Each change in input cost of $4 per ton results in a change of about 5 cents per liter in cost of production, each change of $20 per ton in the soymeal price translates into a change of 1.4 cents per liter in cost of production. Costs are not as sensitive to other financial parameters. Changes to the opportunity cost charged for capital or the amortization period do not add dramatically to cost. Adding another $10 million to the investment would add about 2.5 cents per liter to the cost of production.

The impact of the plant size on the final production costs per liter was also investigated. The total cost of ethanol before by-product credits is $0.47 per liter of which about 18 cents are direct inputs for which there would be little size effect. If the remainder ($0.29 per liter) is scaled by size of plant, the changes in the production cost per liter according to plants of different sizes would be as in Tab. 45.

Tab. 46. Overall Energy Balance for Grain Alcohol Production from Corn (Scheller and Mohr, 1976)

Energy Production	kJ per 35.42 L of Corn	kJ per L of Ethanol
Ethanol	206,698	21,052
Aldehydes, fusel oil	2,953	300
Stalks, cobs, husks	453,354	46,525
Total	663,005	67,525
Energy consumption		
Farming operation	125,731	12,805
Transportation of stalks, etc.	4,265	434
Alcohol plant	295,078	30,052
Total	425,074	43,291
Net energy production	237,932	34,234
Net energy loss		
By-product grain production	49,664	5,060

Tab. 47. Use of Petroleum Type Energy for Growing Corn and for Alcohol Production (Schruben, 1980)

Input	Today [kJ L^{-1}]	In One Year[a] [kJ L^{-1}]	Under study[b] [kJ L^{-1}]
To grown corn	9,688	9,688	9,688
To cook and convert	3,990	3,990	3,990
Germ recovery	624	624	624
Distilling	7,906	7,906	5,534
Gluten recovery	731	731	731
Feed recovery[c]	7,303	4,416	4,416
Total electrical energy	1,061	1,061	1,061
Total per liter	31,303	28,416	26,044
Substract electrical energy[d]	1,061	1,061	1,061
Petroleum type energy input	30,242	27,355	24,983
Output[e]			
Alcohol (if LHV)	21,052	21,052	21,052
Alcohol (if HHV)	23,291	23,391	23,391
Net balance for this plant			
(if LHV)	−9,190	−6,303	−3,931
(if HHV)	−6,851	−3,964	−1,592
Liters petroleum energy equivalent used for each liter of alcohol produced			
(at LHV)	1,436	1,299	1,187
(at HHV)	1,293	1,169	1,068

[a] By using a recompression evaporator with an electric motor.
[b] By using ether for dehydrating.
[c] If stillage is fed to livestock direct from the distillery, then some of the energy would not be used for feed recovery. However, this alcohol plant does use the energy and since it is not available for other users, it must be included for a proper comparison.
[d] Although recompression equipment is to be powered by electricity, no allowance was made by the distiller for increased use. However, electricity was assumed to be generated using nonpetroleum-type fuels (coal or nuclear) although a portion or all may be generated using oil or natural gas
[e] High heating values (HHV) were used throughout the application which tends to maximize the kJ value of the alcohol input. The low heating value (LHV) is also presented here to provide another comparison.

8.2 Energetics

8.2.1 Ethanol from Corn

The consumed energy for corn and ethanol production and that obtained through products is shown in Tab. 46. A positive energy balance was achieved by taking into account the energy that could be obtained and used in the process through burning of the whole quantity of stalks, cobs, and husks. Scheller and Mohr (1976) proposed for practical reasons to use about 75% cobs, stalks,

Tab. 48. Energetics of Ethanol Production from Sugarcane and Cassava[a] (de Carvalho et al., 1977)

Raw Material	Case	Energy [$4.19 \cdot 10^6$ kJ]					Net Energy Ratio (Output/ Input)
		Input Output	Agri-culture	Distillery	Trans-portation	Total	
Sugarcane	Total on-site generation of electric power (sugarcane bagasse as fuel)	5.59	0.42	0.017	0.26	0.70	8.0
Cassava	External supply of electric power (wood as fuel)	5.57	0.30	0.43	0.21	0.94	5.9
Cassava	Total on-site generation of electric power (wood as fuel)	5.57	0.30	0.045	0.27	0.62	9.0

[a] Basis: 1 m^3 anhydrous ethanol.

and husks and to leave the rest in fields for soil conditioning. In that case, the net energy production is still about 12.58 MJ L^{-1} ethanol if the energy deficit for by-product production is not included, or more than 7.53 MJ L^{-1} ethanol if that deficit is included. This analysis is one of the most optimistic. Removing the stalks, cobs, and husks creates a problem because it means mining of soil, and in a short period of time it would cause environmental problems.

Taking into account that the energy crisis is caused by the shortage of crude oil it is interesting to compare how much petroleum energy is used for growing corn and for ethanol production. These data are shown in Tab. 47. Three different processes are compared. For each process, the petroleum-type energy input is higher than output through ethanol and for each liter of ethanol produced, more than 1 liter of petroleum energy equivalent would be used.

8.2.2 Ethanol from Sugarcane and Cassava

In Tab. 48, energy input and output are shown for production of anhydrous ethanol from sugarcane and cassava in plants with a capacity of 150 $m^3\ d^{-1}$ ethanol. Taking into consideration the energy balance for ethanol production from sugarcane, it is supposed that total electric energy is generated on site and sugarcane bagasse is used as a fuel for steam generation. In the process with cassava, external electric power is used and wood is the fuel source for process steam generation.

Tab. 49. Energy Balance for Conversion of Aspen Wood Chips to Ethanol[a] (Wayman, 1979)

	Gross Energy Recovery by	
	Acid Hydrolysis	Enzyme Hydrolysis [10^6 kJ]
Ethanol	6.84	8.06
Lignin	4.41	4.41
Volatiles	+	+
Total	11.25	12.47
Energy recovery	52.35%	58.03%
Energy required		
Autohydrolysis	0.70	0.70
Caustic extraction	0.12	0.12
Hydrolysis	0.70	0.23
Distillation	1.97	2.32
Total	3.49	3.37
Net energy recovery	36.1%	42.3%

[a] Basis: 10^3 kg of aspen wood chips (dry); heat of combustion of aspen chips: $21.48 \cdot 10^6$ kJ.

Tab. 50. Energy Balance for Conversion of Cornstalks to Ethanol (Sitton et al., 1989)

Item	Heat Available [10^6 kJ h^{-1}]	Temperature [°C]	Heat Required [10^6 kJ h^{-1}]
Acid mixing in first hydrolysis tank	5.80	100	–
Acid reaction in impregnator	1.84	80	–
Acid mixing in second hydrolysis tank	1.42	110	–
Feed to second hydrolysis	–	80–110	5.80
Cooling feeds to fermenters	56.39	110–25	–
Heating feeds from fermenters	–	25–90	48.90
Heat supplied to reboiler	–	100	35.20
Heat recovered from condenser	7.38	84	–
Energy from remaining solids	39.00	–	0.84
Energy required for pumps and centrifuges	–	–	–
Total	111.83		90.74

8.2.3 Ethanol from Wood

If we consider the experimental production of ethanol from aspen wood chips (Wayman et al., 1979) following autohydrolysis and caustic extraction, the ratio of output/input energy is 3.2 and 3.7 for a process assuming acid hydrolysis and enzymatic hydrolysis, respectively (Tab. 49). It can also be seen from the energy balance that all energy required to operate the process can be supplied by the lignin that is recovered. For this calculation the author assumed a 95% yield of ethanol from wood sugars obtained after hydrolysis.

8.2.4 Ethanol from Cornstalks

Corn stover is a feedstock material available in high volume as a by-product from today's corn. The requirement and availability of energy in the process for the production of ethanol (96%) through acidic conversion of cornstalks to sugars following fermentation and distillation are presented in Tab. 50. A production of 1,708 kg h^{-1} ethanol is taken as a basis for this energy balance. A positive energy balance was obtained assuming that almost all necessary energy can be supplied from fermenters (cooling) and from remaining solids. A net energy yield of this process is the ethanol produced (2,165 L h^{-1} or 1.26×10^9 kJ d^{-1}).

9 Ethanol as a Liquid Fuel

Bioethanol, in general terms, is a fuel-grade ethanol made by fermentation. It represents a sustainable substitute for gasoline in today's passenger cars. Modeling and design of processes for making bioethanol are critical tools used in bioethanol research and development program. Such analysis is used to guide new directions for research and to facilitate the understanding of the level at which and the time when bioethanol will achieve commercial success. A comprehensive study of ethanol as a liquid fuel was conducted by Kosaric (1984). Foreign crude oil imports currently provide the raw material for the production of half of the liquid fuels consumed in North America (U.S. Department of Energy, 1980). Recent events have dramatically illustrated the considerable economic cost, instability, and economic vulnerability of such imports. Even though oil prices have recently fallen, there is no security in believing that they will stay at these levels. Ethanol is a liquid fuel that can substitute for some petroleum products now, and increasingly so in the future years. As crude oil price increases above US$ 30 per barrel, bioethanol as a fuel becomes very attractive, as it is renewable and economically sustainable. There is a lot of research done in the U.S., in Brazil, and in Canada to utilize ethanol as a fuel.

A concerted effort on behalf of government and the private sector is necessary to promote an ethanol-based fuel industry. Legislation, specifically dealing with programs to set aside land, to allow for the cultivation of biomass on marginal lands, is an essential element to developing this industry.

In Brazil, an effort of government and the private sector was launched in 1975 to develop an alcohol fuel industry. OPEC's 1973 price hike increased Brazil's debt burden significantly. Petroleum products, of which more than 80% were imported, accounted for about 42% of the nation's energy consumption and more than 50% of foreign exchange outlays (Renewable Energy News, 1983).

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The Brazilian government launched a program called PRO-ALCOOL, and encouraged the private sector to play a key role in the development of alcohol production technology. Consequently, from 1975–1980, 300 private distilling projects received 90% start-up financing (Pischinger and Pinto, 1979).

During the same time period, 10 regional technology research centers collaborated with domestic automobile manufacturers to perfect an efficient alcohol burning engine. Engine conversion technologies were transferred to 500 private enterprises throughout Brazil and mass production of alcohol vehicles was started in 1979. The Brazilian effort showed promise, as 250,000 alcohol burning vehicles and 80,000 automobiles converted to run on a 20% ethanol–gasoline mixture, became functional in the transportation sector.

Even with such advances, certain problems became clear and evident: The alcohol burning engines did not meet the requirements of long-term operation, and there was serious doubt whether ethanol production would meet the demands of the transportation sector. The government provided an aide package to producers for $1 billion, and a research effort with the major car manufacturers to develop a second generation alcohol burning engine. These measures caused an increase in alcohol vehicle sales by 30%, and increased ethanol production by 62% (Conceiro, N.P., Head Powertrain Engineering, General Motors do Brasil S.A., personal communication, 1983).

In Canada, alcohol fuels are commercially available in most of the provinces. Since 1981 a 10% ethanol–gasoline blend is available at approximately the same price as regular gasoline (Renewable Energy News, 1983).

In Ontario, there is presently a revived interest to develop an industry to supply fuel ethanol. Ethanol production and use is likely to be initiated by the farming sector in Ontario. Farms can produce ethanol because they have the raw material for its synthesis. The ethanol can be used in farm diesels like trucks, tractors, and combines. On-farm production of fuel gives the farmer:

- insurance of fuel supply,
- ability to use wet, spent grains, and
- investment and fuel tax brakes.

Ethanol production technology is advanced to the point that small-scale ethanol plants have been designed with "step-by-step" instructions for the prospective producer (Berglund and Richardson, 1982; Chambers et al., 1979; Gird, 1980; Leeper et al., 1982; Lincoln, 1980; McAtee et al., 1982).

Canada, with its vast land area and agricultural potential, is in a position to provide the necessary raw material(s) for ethanol production. The particular crop(s) that might be chosen will depend on many economic variables. However, it is evident that the agricultural sector has a role whose potential is just beginning to be realized. A farm-based ethanol fermentation industry can provide a starting point for future large-scale ethanol production.

9.1 Characteristics of Ethanol and Gasoline–Ethanol Blends as Motor Fuel

It is interesting that gasoline and alcohol, which in many respects are quite different, have a nearly equal energy of combustion per unit volume of stoichiometric mixture. It may, therefore, be concluded that these fuels used in an engine, under the same conditions, with the same fraction of the stoichiometric mixture for both, and with fully vaporized fuel, will provide nearly the same power. Hence, the power of an engine cannot be significantly altered by changing these fuels, for similar charge conditions. Equal amounts of energy and power require the use of an approximately 60% greater weight of ethyl alcohol than gasoline (Bolt, 1980).

The vapor pressure of alcohol is greater than that of gasoline, and the latent heat of vaporization is higher, which is primarily responsible for the increased power outputs using alcohol. As a result of the lower vapor pressure and high latent heat of vaporization, it is harder to start a cold engine on ethanol than on gasoline. To correct this deficiency, it has been common to add ether, benzene, or gasoline to the alcohol, each of which serves to increase the vapor pressure, or simply start the engine with gasoline and then switch to alcohol.

A variety of fuels were tested using a Ricardo single cylinder internal combustion engine by varying the fuel compression ratio. Fig. 51 is a plot of the test results for 198 proof ethyl alcohol and gasoline. The increased mean effective pressure obtained using ethanol at all mixture ratios is the most dramatic difference between the two fuels (Menrad and Loeck, 1979). The increase in pressure is principally due to the greater volumetric efficiency, which results from the high latent heat of vaporization of alcohol and the greater mass of fuel per unit mass of air. Specifically, the higher latent heat of vaporization of ethanol as compared to gasoline produces a greater lowering of temperature upon evaporation, in the intake manifold, resulting in a cooler and denser fuel–air mixture. Hence, a greater charge of air and fuel is taken into the cylinder during the suction stroke and the volumetric efficiency, as mentioned above, increases.

9.1.1 Exhaust and Evaporative Emissions

Nitrogen oxides are produced during the combustion process and increase with higher temperatures. The lower flame temperature of ethanol produces substantially less NO_x than hydrocarbon fuels, at any equivalence ratio. When alcohol is used in gasoline as a blend, a reduction in NO_x emissions roughly proportional to the alcohol concentration is observed (Bechtold and Pullman, 1979; McCallum et al., 1982). When no adjustment of the fuel metering system

is made, alcohol–gasoline blends may either increase or reduce NO_x emissions, depending on whether the blend leaning effect moves the fuel–air equivalence ratio closer or further from the approximate value of 0.9, at which NO_x typically peaks. The change is usually slight for moderate blend levels.

Fuel evaporative emissions from alcohol blends are typically much higher than those from straight gasoline (General Motors Research Laboratories, 1980). A 10% ethanol–gasoline blend may increase evaporative emissions of 49% to 62% relative to straight gasoline (McCallum et al., 1982).

One major exhaust problem from the use of ethanol is that of aldehyde emissions (especially acetaldehyde), which are not currently regulated. Aldehydes are photochemically reactive and highly irritating to mucous membranes. Aldehyde emissions from neat ethanol may be from 2–4 times of those from gasoline (Wagner, 1980).

Carbon monoxide emissions are usually reduced when using ethanol–gasoline blends. This is not directly the result of the ethanol fuel, but rather the fact that CO emissions – and unburned fuel emissions – decrease when combustion occurs at lean equivalence ratios. Therefore, the lower CO emissions associated with ethanol–gasoline blends are mainly due to the blend leaning effect. It may be generally stated that with alcohol fuels, CO emissions are not reduced to the same extent as NO_x emissions, and are similar to those from gasoline powered engines (Menrad, 1979).

9.1.2 Ignition, Cold Start-Up, and Driveability

Partial-load operation shows that alcohol-operated engines require less ignition advance than gasoline engines (Menrad, 1979). This is mainly due to the higher compression ratio that can be attained with alcohol fuels, and the higher rate of flame propagation.

The most frequently encountered difficulty with ethanol fuel is the poor cold start-up of the engine, especially evident in northern climates during winter. The failure of engines operating on ethanol to start under cold ambient conditions is a direct consequence of the inability of the initial spark ignition energy to produce a self-propagating flame with the required mass burning velocity (Rajan, 1979).

To alleviate the problem of cold start-up, a fuel vaporizer has been developed to facilitate cold starting of ethanol-fuelled engines. The vaporizer ultimately found to give the best results was designed with a double concentrically wound cell. This design enables vehicle cold starts at temperatures as low as $-10\,°C$.

Generally, the poor driveability characteristics of some ethanol–gasoline mixtures is related to an intolerable leaning of the fuel–air ratio. This might occur by the simple mechanism of the blend leaning effect. Newer vehicles

equipped with feedback-fuel metering systems maintain a constant fuel–air stoichiometry, and the results of the leaning effect are minimized.

9.1.3 Water Tolerance of Ethanol–Gasoline Blends

Most alcohol is not totally pure and contains 5% water. This is due to the fact that ethanol forms a low-boiling azeotrope during distillation and the 5% water content cannot be separated out. Since distillation cannot increase the concentration of ethanol directly, the addition of benzene forms a ternary mixture of benzene, water, and ethanol with a lower boiling point of 64.85 °C, from which the water can be removed by distillation. Benzene and alcohol have a low- boiling binary (67.8 °C), hence benzene can be separated by distillation. Anhydrous ethanol is obtained commercially in this manner as 200 proof ethanol.

Gasoline and anhydrous ethanol are miscible in all proportions and over a wide range of temperatures. However, even small amounts of water in a gasoline–ethanol blend will cause separation of the ethanol and gasoline. Fig. 52 shows the water tolerance of alcohol–gasoline blends over a wide temperature range. A 20% alcohol–gasoline blend at room temperature can, at most, tolerate 0.3% water before phase separation. Certain blending agents can be added to the mixture which will increase the water tolerance. Some of these include benzene, acetone, and butyl alcohol (Bolt, 1980; Terzoni, 1981).

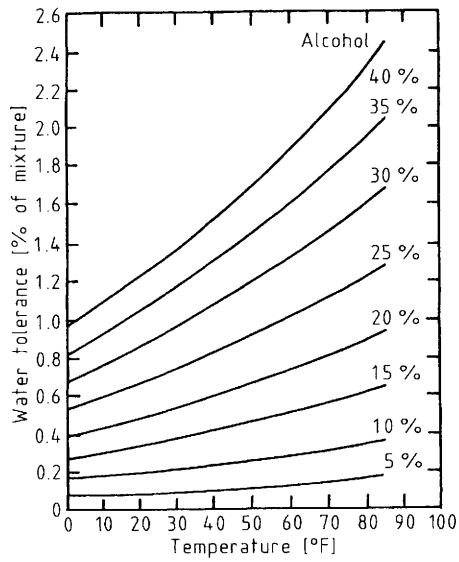

Fig. 52. Water tolerance of alcohol–gasoline blends.

9.1.4 Lubrication

A problem which may result in any alcohol-fuelled engine is dilution of the lubricating oil with alcohol condensed from blow-by gases or that which finds its way past the piston rings. This can have adverse effects on the wear of bearings, camshafts, and other parts which rely greatly on lubrication to function properly.

It is known that both gasoline and diesel fuel have some lubricating quality, while ethanol has none. Alcohol presents a particular problem since it is known that it has a tendency to wash cylinder walls. This would not be a problem if it could be ensured that ethanol stays in its vapor state during operation of the engine.

Oil of the castor bean is one of the few lubricants that blends with alcohol. 5 vol.% castor oil in ethanol provides the same lubrication as diesel fuel (Kirik, 1981). However, castor oil and ethanol do not mix easily and vigorous mixing is required. Furthermore, castor oil solidifies between 0 °C and 30 °C, and hence might present problems in cold climates.

9.1.5 Corrosion and Materials Compatibility for Alcohol-Fuelled Vehicles

Little is known about the wear of ethanol-fuelled engines. At present, the most likely explanation seems to be that a combination of corrosion and erosion is causing wear, though other mechanisms may be present (Mueller Associates Inc., 1981). When ethanol burns with insufficient air, acetic acid is formed, which promotes corrosion in the engine (Kirik, 1981); the smell of vinegar in the exhaust can be used as a signal to adjust the fuel–air ratio.

Ethanol has adverse effects on some gasket materials, certain rubber materials, and some metals. It acts as a good cleaning agent for the deposits left behind by gasoline and for rust. If ethanol is stored in a tank which previously held gasoline, it can be expected that any deposited residue will be removed from the tank walls. This means that fuel filters in dispensing equipment and vehicles may become restricted or plugged soon after the change of fuels. The initial cleansing activity loosens the majority of the material which will be removed and, thus, residue loosening is less a problem as time goes on.

Steel is not greatly affected by ethanol. However, if the water content in an alcohol–gasoline blend is high enough to cause phase separation, the steel in contact with the water-rich phase will show corrosion.

Anhydrous ethanol and ethanol–gasoline blends exert adverse effects on most elastomeric parts such as fuel-pump diaphragms and fuel hoses. These parts deteriorate gradually making more frequent replacement than with gasoline necessary. Fiberglass shows softening and blistering when coming in contact with ethanol, indicating destruction of the laminate. Polyurethane is sof-

tened and cracked by contact with ethanol. Nylon, high-density polyethylene and polypropylene are not affected significantly by ethanol.

Elastomers swell and shrink in any organic liquid depending on the particular elastomer and the organic liquid. Therefore, it can be expected that many parts made of elastomers will behave differently if ethanol is used in a fuel system designed for gasoline or diesel fuel.

9.1.6 Safety of Alcohol

In general, ethanol is considered to be less dangerous than gasoline or diesel fuel (Mueller Associates Inc., 1981). Since alcohol does not contain light fractions, alcohol fires do not start as readily as gasoline fires. However, alcohol burns with a nearly invisible flame, especially in sunlight, and without the large amount of smoke generated by gasoline. Therefore, detection of ethanol fires might be more difficult.

Due to the high vapor pressure of ethanol, flammable mixtures of air and anhydrous ethanol fuel vapors will be present in fuel tanks under most ambient conditions. Alcohol and air mixtures of 4–13.6% alcohol are explosive; the range for gasoline being only 2–5% (Kirik, 1981). To circumvent this problem, collapsible fuel tanks can be made, as is already done for safety in racing cars.

9.2 Modifications and Conversions of Existing Internal Combustion Engines to Utilize Ethanol and Ethanol–Gasoline Blends

9.2.1 Research

The utility of alcohols as substitute fuel for the internal combustion engine has been under investigation for some time. As early as 1897, Nikolaus A. Otto used pure ethanol in his first engine (Rothman, 1983). In the 1920s and 1930s in Germany, Switzerland, and in the U.S., fuel ethanol was used extensively. Both, beneficial and detrimental characteristics of ethanol as a motor fuel have long been noted and widely recognized.

The General Motors Research Laboratories in Warren, Michigan, have conducted research using ethanol fuel in a single-cylinder engine, similar to the Ricardo E.6/T engine. The researchers found that engine thermal efficiency increased by 3% with ethanol, compared to gasoline at the same compression ratio (Brinkman, 1981). Furthermore, increasing the compression ratio from 7.5:1 to 18:1 with ethanol increased efficiency by 18% over gasoline.

The U.S. Department of Energy conducted research on the influence of a 20% ethanol–indoline blend on the steady-state performance and emission

characteristics of a carburated spark-ignited engine. Data obtained revealed that a 2% increase in engine torque was observed with the blend and a 1.7% rise in brake thermal efficiency, compared to the base fuel. CO emissions remained the same while NO_x and HC emissions decreased with the blend, compared to the base fuel (indoline). From the results obtained, it was concluded that for the same engine conditions, the substitution of practical level alcohol blends (10–15% by volume) will have little effect on steady-state performance and currently regulated emission characteristics (CO, HC, and NO_x) (Adt and Rhee, 1978).

The Coordinating Research Council Inc. (C.R.C.I.) conducted tests to determine the effects of 10% ethanol–gasoline blends on emissions, fuel economy, and driveability of 14 1980-model year cars. They found that a 10% ethanol addition to gasoline resulted in statistically significant changes in emissions, driveability, and efficiency (Bernstein et al., 1982).

Exhaust and evaporative emissions from a 1974 Brazilian Chevrolet Opala were measured (Furey and Jackson, 1977) using gasoline and various ethanol–gasoline mixtures. A 20% ethanol–gasoline mixture reduced exhaust hydrocarbon and CO emissions, but increased aldehyde and NO_x emissions. It appears that leaning of the air–fuel mixture, due to ethanol addition, was the primary cause of the exhaust emission changes.

The U.S. Environmental Protection Agency conducted evaporative, exhaust, and performance characteristics on 11 test vehicles and found a 2% decrease in fuel economy (Furey and King, 1980; Lawrence, 1979). The Japan Automobile Research Institute has demonstrated that NO_x emissions decrease in I.C. (international combustion) engines as the ratio of alcohol in gasoline increases (Matsuno et al., 1979).

Nebraska, which was one of the first states to implement GASOHOL (10% anhydrous ethanol with regular-grade unleaded gasoline), used 45 vehicles in a 3.2 million km road test to compare GASOHOL with regular-grade unleaded gasoline. GASOHOL cars obtained up to 5.3% more miles per gallon, and 8.07 more miles per Btu than the cars using unleaded fuel (Scheller, 1977). GASOHOL performance was satisfactory under all conditions of weather and driving.

Kosaric (1984) studied combustion of varying gasoline–ethanol and ethanol–water fuel blends using a Ricardo E.6/Y variable compression, spark-ignited engine at compression ratios ranging from 8.5–15.8. The investigation revealed that the engine could be operated at considerably higher compression ratios when ethanol fuels were used instead of gasoline fuels. Neat ethanol compared to straight gasoline had a 25% higher power output (at 1,600 rpm and CR 8.5 : 1); the power output obtained using neat ethanol at CR12.5 and 1,600 rpm represents a 57% increase relative to straight gasoline at CR 8.5:1, and a 26% increase relative to neat ethanol at CR 8.4:1.

The best fuel economy was obtained with gasoline–ethanol blends at high compression; the lowest fuel consumption of all blends tested was recorded us-

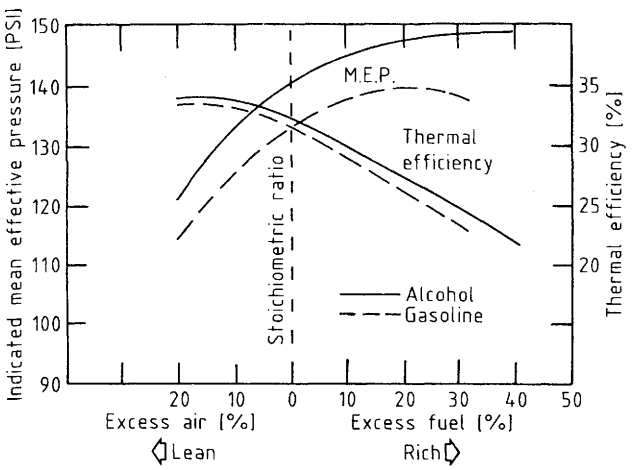

Fig. 53. Engine performance with ethyl alcohol and gasoline.

ing a 40% ethanol–gasoline blend at CR 11.9:1. Ethanol–water fuel mixtures generally showed higher thermal efficiencies than gasoline–ethanol fuel blends; a 36% increase in thermal efficiency was obtained using neat ethanol relative to straight gasoline, at CR 8.5:1. Due to the performance characteristics obtained, ethanol with a 20% water content was recommended as the fuel blend of choice for operation in gasoline-powered engines at low compression (8.5:1) (Fig. 53).

9.2.2 Applications

Gasoline powered engines can be modified to run on ethanol or ethanol–gasoline blends. Furthermore, some car manufacturers such as Volkswagen, General Motors, Volvo, and Daimler Benz have designed and developed alcohol engines. As might be expected, such conversions have varying levels of sophistication.

The main principle of the simple conversion is to aid in the vaporization of ethanol by starting the engine with gasoline and switching to ethanol after about 5 min running time with gasoline. A second fuel tank for ethanol can be used and an appropriate mechanism to switch from the gasoline to the ethanol tank incorporated into the fuel delivery system of the engine. Some conversion systems are offered commercially. The diameter of the main metering jet of the carburetor needs to be increased by 25% due to the higher volumetric flow of ethanol (1.5) relative to gasoline and, therefore, the fuel–air ratio needs to be appropriately adjusted. The resulting engine power is approximately the same as that for gasoline.

An improved conversion uses high compression as well as some of the above mentioned modifications. Power output is increased by about 10% over a similar gasoline engine, and ethanol consumption is only 10–20% higher by volume (Kirik, 1981). Some of the changes include fitting new piston heads to increase the compression ratio to 12.5:1, and fitting larger carburetor jets to accommodate for the increased alcohol volume.

Starting from cold is similar to that of the simple conversion, but gasoline as a starting fuel is not recommended; an alcohol–ether mixture from 15–50% ether by volume, might serve as a starting fluid. Propane can be injected for cold starting, or one of the external heat sources can be applied to vaporize alcohol.

9.3 Comparison of Ethanol with Other Motor Fuels

A comparative study of methanol, ethanol, isopropanol, and butanol as pure gasoline-blended motor fuels was presented by Kelkar et al. (1989). An International Harvester Silver Diamond engine (Tab. 51) was used for testing different fuels. The cooling arrangement of the engine was modified by removing the radiator and water pump and the carburetor was fitted with a load needle rather than a fixed jet. To ensure enough alcohol supply to the engine, the main jet tube was redrilled to give 35% increase in area.

The experimental results showed that a 10% alcohol–90% gasoline mixture may be used in engines designed for gasoline without modifications. Gasoline blends up to 50/50 with any of the alcohols tested may be used if carburetor adjustment and ignition timing modifications are made to the engines designed for pure gasoline. However, a spark-ignition engine designed to use gasoline must be modified to use pure alcohol. Changes required for the use of methanol, ethanol, isopropanol, and butanol use are technically similar, but the fuels could not be used interchangeably without carburetor adjustment. The basic engine changes included an increase in the compression ratio, enlarging the carburetor jets, and adjusting the ignition timing.

Tab. 51. Specifications for the International Harvester, Silver Diamond Testing Engine (Kelkar et al., 1989)

Engine Type	Reciprocating, Spark Ignition Engine
Number of cylinders	6
Cylinder arrangement	in-line, vertical
Bore × stroke	3 9/16″ × 4″
Displacement	240.00 cubic inches compression
Ratio	1. original head: 6.77:1 2. modified head 7.76:1
Type of cooling	water-cooled

A comparison of production costs of liquid fuels from biomass has been presented by Bridgewater and Double (1991, 1994). A computer-aided economic analysis is presented for the following processes:

- Mobil's Methanol to Gasoline (MTG) (Lurger gasifier),
- gasoline from wood by the Mobil's Methanol to Olefrus Gasoline and Diesel (MOGD),
- diesel from wood by SMDS (Shell Middle Distillate Synthesis),
- methanol from wood by gasification,
- fuel alcohol from wood (blend of methanol with higher alcohols produced by a modified methanol synthesis process),
- direct thermochemical conversion of wood to gasoline blending stock,
- ethanol from softwood (fermentation of hydrolyzate),
- methanol from RDF (Reception of Refuse-Derived Fuel),
- methanol from straw,
- ethanol from straw (fermentation of hydrolyzate),
- ethanol from beet (fermentation),
- ethanol from wheat (fermentation).

The absolute cost of fuels at 1,000 t d^{-1} feed rate are summarized and ranked in Tab. 52 with feed costs at typical values.

Tab. 52. Estimated Fuel Cost[a] (Bridgewater and Double, 1994)

Rank	Product	Feed	Route	Uncertainty	Cost [£ GK^{-1}]
	Natural gas				3.0
	Diesel				4.1
	Methanol				5.6
	Gasoline				5.8
1	Methanol	straw	gasification	low-moderate	7.0
2	Gasoline	wood	pyrolysis + zelolites	high	8.6
3	Diesel	wood	gasification + SMDS	low-moderate	8.8
4	Gasoline	wood	pyrolysis + hydrotreating	high	8.8
5	Methanol	wood	gasification	low-moderate	9.1
6	Gasoline	wood	liquefaction + hydrotreating	high	9.4
7	Methanol	RDF	gasification	moderate	9.9
8	Fuel alcohol	wood	gasification	moderate	9.8
	Ethanol				12.0
9	Gasoline	wood	gasification + MTG	low	12.4
10	Ethanol	wood	H_2SO_4 hydrolysis + fermentation	low	14.4
11	Gasoline	wood	gasification + MOGD	moderate	14.6
12	Diesel	wood	gasification + MOGD	moderate	14.6
13	Ethanol	wheat	fermentation	low	14.9
14	Ethanol	wood	enzyme hydrolysis + fermentation	moderate	19.6
15	Ethanol	beet	fermentation	low	25.2

[a] Basis: 1,000 t d^{-1} daf feed, feedstock costed at typical cost.

In terms of absolute fuel costs, thermochemical conversion offers the lowest cost products, with the least complex processes being advantageous. Biochemical routes, according to this analysis, are the most costly in absolute terms.

The most attractive processes, by comparing production costs to product values, are alcohol fuels. Direct production of highly aromatic gasoline by direct liquefaction of wood through pyrolysis and zeolites is better, but the process is so far the least developed. For hydrocarbons, the direct liquefaction processes appear to offer considerable advantages, although the state of development is less advanced.

Of the indirect thermochemical routes, the MTG appears to be the most attractive for gasoline and the Shell SMDS for diesel. Both of these processes also have the advantage of producing high-quality products: MTG gasoline is highly aromatic (high octane number), while SMDS diesel has a octane number far in excess of current specifications, allowing engines on this fuel to be much more efficient than current diesels. The SMDS process has the advantage of producing jet fuel of high quality.

10 Present and Potential Markets for Ethanol

In the United States alone, fuel alcohol has grown from its infancy in 1979 to approximately 11.10^9 L (2.9×10^9 gallons) of production capacity in 1991 (Haigwood, 1991). Its price was based on the price of wholesale gasoline plus available federal state tax incentives. These incentives allowed ethanol, with production costs of $0.26–0.40 per liter ($1.00–1.25 per gallon) to compete with gasoline prices of $0.11–0.17 per liter ($0.40–0.65 per gallon).

Internationally, ethanol fuels have been widely used. In the United States of America, sales of ethanol–gasoline blends represent about 8–9% of the total gasoline sales, which is actually greater than the entire Canadian gasoline market. It is estimated that Americans have driven over 3 trillion kilometers on ethanol-blended fuels since 1979. More than 11 billion liters of ethanol for fuel are produced each year in Brazil, where about 15% of vehicles with spark ignition engines are run on neat ethanol and the rest use a blend of 20% of ethanol in gasoline. In Canada there are over 700 ethanol gas outlets across the country.

Woodley et al. (1999a) provide an update on our latest estimates for current and projected costs of bioethanol (Tab. 53). These estimates are the result of very sophisticated modeling and costing efforts undertaken in the program over the past few years. Bioethanol could cost anywhere from $1.16 to $1.44 per gallon, depending on the technology and the availability of low-cost feedstocks for conversion to ethanol. While this cost range opens the door to fuel blending opportunities, in which ethanol can be used, e.g., to improve the oc-

Tab. 53. Parameter Changes in the Future (Woodley et al., 1999b)

Process Area	Parameter	BaseCase	Best of Industry	Year 2005	Year 2010	Year 2015
Cost $/gal		$1.44	$1.16	$0.94	$0.82	$0.76
Feedstock	cellulose fraction	42.67%	42.67%	42.67%	42.67%	51.20%
	xylan fraction	19.05%	19.05%	19.05%	19.05%	22.86%
	arabinan fraction	0.79%	0.79%	0.79%	0.79%	0.95%
	mannan fraction	3.93%	3.93%	3.93%	3.93%	4.72%
	galactan fraction	0.24%	0.24%	0.24%	0.24%	0.29%
	lignin fraction	27.68%	27.68%	27.68%	27.68%	14.34%
Pretreatment	reactor residence time [min]	10 min	8 min	8 min	8 min	8 min
	acid concentration	0.5%	1.5%	1.5%	1.5%	1.5%
	temperature [°C]	190	150	150	150	150
	xylan to xylose yield	75%	85%	85%	85%	85%
	mannan to mannose yield	75%	85%	85%	85%	85%
	galactan to galactose yield	75%	85%	85%	85%	85%
	arabinan to arabinose yield	75%	85%	85%	85%	85%
	type of conditioning [a]	IX/OL	IX/OL	OL only	OL only	OL only
Enzyme production	enzyme loading for SSCF [b]	15 FPU g^{-1}	15 FPU g^{-1}	15 FPU g^{-1}	20 FPU g^{-1}	20 FPU g^{-1}
	yield [FPU g^{-1} cellulose + xylose]	200	200	600	2,000	2,000
	productivity [FPU $L^{-1} h^{-1}$][c]	75	200	600	2,000	2,000
	aeration [vvm]	0.577	0.577	0.29	0.29	0.29
SSCF	residence time	7 d	7 d	3.5 d	2 d	2 d
	cellulose to glucose yield	80%	80%	80%	90%	90%
	glucose to ethanol yield	92%	92%	92%	95%	95%
	xylose to ethanol yield	85%	85%	85%	95%	95%
	arabinose to ethanol yield	0%	85%	85%	95%	95%
	galactose to ethanol yield	0%	90%	90%	95%	95%
	mannose to ethanol yield	0%	90%	90%	95%	95%
	temperature [°C]	30	30	55	55	55
	chilled water fraction	20%	20%	0%	0%	0%
	contamination loss	7%	7%	5%	3%	3%
Utilities	biomass to burn flow [dry kg h^{-1}]	0	0	0	0	11.000
	biomass to burn [dry ton d^{-1}]	0	0	0	0	275
	on-line time [h a^{-1}]	8.406	8.406	8.406	8.406	8.478

[a] IX: Ion exchange, OL: overliming [b] SCF: Simultaneous saccharification and co-fermentation [c] FPU: Filter paper units

tane rating of gasoline, it is not currently competitive with gasoline as a bulk fuel. Research strategies and goals described by these workers have been translated into cost savings for ethanol. The analysis of these goals shows that the cost of ethanol could drop by 40 cents per gallon over the next 10 years by taking advantage of exciting new tools in biotechnology that will improve yield and performance in the conversion process.

Even though the cost of ethanol production by fermentation is still too high to openly compete (without subsidies) with gasoline, the environmental factors are of primary importance for development of this industry. The ecological impacts of ethanol fuels for the internal combustion engines can be summarized as follows:

- CO_2: Bioethanol is a renewable energy which does not contribute to the greenhouse effect. In this case, the carbon cycle is very short because the CO_2 given off by fermentation and combustion is reabsorbed by the plants through photosynthesis to vegetation (reduction may be 2- to 3-fold).
- CO and HC: It was shown (Poitrat, 1994) that mixtures with 5–7% ethanol can reduce CO emissions in a proportion of 15–40%. HC emissions may be reduced in a proportion of 2–7%.
- VOC: Ethanol reduces VOC emissions from automobile exhaust. Studies have shown that by reducing VOC and CO emissions, ethanol can help to reduce urban ozone more than any other fuel.
- Particulate matter (PM10): Particulate matter with a diameter of less than 10 μm is regulated by the EPA as small airborne particles can reach the lower regions of the respiratory tract and damage the lung tissues and cause various diseases. It was shown that the use of ethanol as fuel for the internal combustion engines reduces tail pipe emissions of M10 and thus reduces particulate air pollution.
- SO_x: Biofuels do not contain sulfur and do not contribute to acid rain.
- NO_x: Biofuels combustion may increase nitrogen oxides in the exhaust by up to 10%. However, lower combustion temperatures and optimum engine tuning can lead to a drop in NO_x emissions.
- Aromatics: Ethanol does not contain aromatics (unlike some gasolines which contain up to 45% aromatics).
- Aldehydes: Emissions of certain aldehydes/ketones are slightly increased, among which is acetaldehyde. Vehicles equipped with a catalytic converter have shown reductions in aldehyde emissions.
- Nitrates: Maintaining a cover of vegetation during the winter months limits the risk of leaching out fertilizing elements contained in the soil, particularly nitrates.

Maintaining or creating employment by the development of a biofuels industry is also an incentive for development of this industry. Depending on the

feedstock used 10–17 direct or indirect employees could be created or maintained per 1,000 t of ethanol production capacity. The agricultural area per employee would be 19–20 ha for sugar beet as feedstock and 30–35 ha for wheat. The ethanol produced per employment would be 100 t with sugar beet and 60–67 t with wheat.

In addition to the use of ethanol as a pure or gasoline-blended fuel for internal combustion engines, ethanol can also be used in many other very important industrial applications (Haigwood, 1991), such as for:

- Coal desulfurization: In this process, ethanol is added to finely ground coal. The coal is heated and treated with ethanol in the presence of a reaction accelerator. This process removes both the organic and mineral sulfur in a single step, at up to 90% efficiency. The market for this process alone can generate a demand in the USA of over 3.78×10^9 L (1×10^9 gallons) of ethanol.
- Biodegradable plastics: The use of ethanol in the production of biodegradable plastics could create a substantial market for ethanol and, at the same time, reduce the cost of manufacturing biodegradable plastics. Ethanol is used here as a substrate for microorganisms which produce either polyhydroxy butyrate (PHB) or polyhydroxy valerate (PHV). When combined, the two create PHBV, which is a biodegradable plastic. Ethanol as a carbon source in the process could reduce the cost from $33.04 to 2.64 per kg if fuel ethanol excise tax subsidies are applied.
- Cattle feeding: There is indication that dietary ethanol improves the tenderness of beef without adding to the fat content. This could help the cattle livestock industry to produce tender "low-fat" beef. It has been estimated that an ethanol market of $1{,}900 \times 10^6$ L (500×10^6 gallons) per year could be created in this way.
- Diesel engine: Research in heat (100% ethanol) fuel alcohol engines is progressing, whereby ethanol is injected with diesel fuel into the engine in presence of peroxides, which allow the blending of ethanol and diesel. Federal emission standards (for buses and trucks) are easier met with these blends.

11 Future Trends and Research

With the objective to improve efficiency and yields in processes for ethanol fermentation, there is a consensus that the prospects for enzyme improvement through protein engineering are very good. Some of the important trends may be summarized in the following:

- Increased thermal stability of enzymes: Simply by increasing the temperature at which these enzymes can operate, we can dramatically improve the rate of cellulose hydrolysis. The genetic pool available in laboratories

around the world includes thermotolerant, cellulase-producing organisms that represent a good starting point for engineering new enzymes.

- Improved cellulose binding domain: Cellulase enzymes contain a catalytic domain and a binding domain. Improvements in the latter will lead to more efficient interaction between the soluble cellulase enzymes and the insoluble surface of the biomass.
- Improved active site: In addition to modifying the binding domain, it is planned to modify amino acid sequences at the active site. Even minor modifications of the enzymes can lead to dramatic improvements in this catalytic activity.
- Reduced non-specific binding: Enzyme that adsorbs on lignin is no longer available for hydrolysis. Genetic modifications of the enzyme will be geared toward adjusting its surface charge to minimize such unwanted binding. In parallel with the protein engineering work, research is expected to be aimed at improving the productivity of the enzyme expression systems. This includes:
  - Developing microbial organisms for high productivity of enzymes.
  - Genetically engineered crops that contain high levels of cellulase enzymes harvested as feedstock.

Research in the past 10 years on ethanol-producing microorganisms has yielded microorganisms capable of converting hexose and pentose sugars to ethanol. These ethanol-producing microorganisms ferment xylose and glucose mixtures to ethanol with high efficiency. This represents a major advance in technology, as previous conversion of pentose sugars by natural yeasts was not industrially attractive. Furthermore, these new ethanologens have eliminated the need for separate pentose and hexose fermentation trains. Substantial improvement in biomass conversion can be achieved by making the following additional improvements in ethanol-producing microorganisms:

(1) ethanol-producing microorganisms capable of producing 5% ethanol at temperatures greater than or equal to 50 °C,
(2) ethanol-producing microorganisms capable of converting cellulose to ethanol.

Woodley et al. (1999a, b) have recently shown that a doubling of the rate of biomass hydrolysis for every 20 °C increase in temperature of saccharification can be expected if *Trichoderma reesei*-like cellulases are used. The development of ethanologens capable of fermentation at temperatures greater than 50 °C can potentially reduce the cost to meet the desired performance at temperatures of 30–33 °C.

The most advanced processing option is one in which all biologically mediated steps (e.g., enzyme production, enzymatic cellulose hydrolysis, and biomass sugar fermentation) occur in a single bioreactor. This process, also known

as direct microbial conversion (DMC) or consolidated bioprocessing (CBP), can be carried out to various extents by a number of microorganisms, including fungi, such as *Fusarium oxysporum*, and bacteria, such as *Clostridium* sp. However, known DMC strains often exhibit relatively low ethanol yields and have not yet been proven effective in handling high concentrations of biomass.

The cellulase enzymes and the fermenting organisms are the major thrusts of our applied research efforts. Integrating these into a complete process is critical to commercial success. Pilot and bench scale optimization of an integrated process is expected to lead to improvements such as:

- optimal yields and operating conditions for the co-current pretreatment step,
- optimal yields and operating conditions for the critical to simultaneous saccharification and co-fermentation step,
- testing of improved organisms and enzymes as they become available.

An optimized and integrated process using cellulase enzymes and co-current pretreatment technology should be available by 2005.

Research on genetic engineering of agricultural crops holds great promise. The need is anticipated for a focused research effort to develop energy crops that contain higher levels of carbohydrates. Increased carbohydrate content provides a direct improvement in ethanol yield.

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