CRC

LIQUID FUEL DEVELOPMENTS

Donald L. Wise

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Liquid Fuel Developments

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CRC SERIES IN BIOENERGY SYSTEMS

Editor-in-Chief
Donald L. Wise, Ph.D.

Fuel Gas Systems

Potential of biomass. Biogas digester design. Use of the upflow anaerobic sludge blanket process in wastewater treatment. Methane from crop-grown biomass. Methane production from agriculture residues by anaerobic digestion in batch and continuous culture. An integrated approach to the anaerobic digestion process. Comparative anaerobic digestion of secondary organic residues. Generation of electric power from biogas. Assessment of secondary agricultural residues: part I; resources available and evaluation for energy recovery. Assessment of secondary agricultural residues: part II, engineering and economic analysis for conversion to methane and/or alcohol fuels.

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Liquid Fuel Systems

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Liquid Fuel Developments

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Bioconversion Systems

Fuels and chemicals from biomass: a role for gene-splicing technology. Lactic acid production by pure and mixed bacterial cultures. Conversion of lignin to useful chemical products. Chemicals from microalgae. Forage crops as chemical feedstocks. Biomass conversion into chemicals using wet oxidation. Technology and economics of chemicals from wood. An integrated anaerobic digestion system for the production of energy and livestock feed based on aquatic biomass production on sand using seawater spray. Liquid fuel production from biomass in the developing countries — an agricultural and economic perspective. Part I: introduction and background. Part II: the tropical environment and the availability of suitable land. Part III: agricultural properties of energy crops. Part IV: economic analysis of liquid fuel options and summary and conclusions.

SERIES PREFACE

This series on Bioenergy Systems grew out of the editor's work on projects sponsored by the Biomass Energy Systems Branch (formerly the Fuels from Biomass Branch) of the U.S. Department of Energy and the editor's professional acquaintance with other workers holding subcontracts from the Solar Energy Research Institute, an organization in Golden, Colorado, totally funded by the U.S. Department of Energy. The U.S. Department of Energy organized the workers holding grants or contracts in biomass into several groups, including one on gaseous fuel production via anaerobic digestion and one on liquid fuel (primarily alcohol) production; the editor was involved with several projects in both groups. The leading workers in these biomass/bioconversion areas of the solar energy field agreed to prepare overviews of their projects for this series of texts. Additionally, workers from around the world were invited to prepare descriptive summaries of their activities in the area of gaseous and liquid fuels production, as well as organic chemicals, from agricultural residues and crop-grown biomass.

This series is a sequel to the two volumes the editor recently had published by CRC Press, Inc. (1981) entitled *"Fuel Gas Production from Biomass"*. These earlier texts described largely the status of methane fermentation and are believed to be the first collected set of reports in which is described all the technical activities in the area of anaerobic digestion with the specific objective of optimizing fuel gas production. It is interesting to note that much of the developmental activities reported on in these earlier texts described the methane fermentation of animal manures — this is simply because manure, as the most readily available and fermentable substrate, was the first to receive attention. Now that some animal manure to fuel gas systems are becoming commercial, research and development attention is being directed towards agricultural residues, especially stover and straws. With these substrates, present attention is focused not only on methane fermentation but on liquid fuel production and even bioconversion to more highly valued organic chemicals. There are an array of readily available organic substrates, but they require a better understanding of processing technology before economic bioconversion to fuels and organic chemicals may be practiced.

In a similar manner as with the earlier texts, these present texts describe the continued efforts in this newly emerging energy recovery field. The major input/output aspects of the bioconversion field are emphasized in this present series of texts. One major aspect of the series on bioenergy systems with respect to inputs of feedstock is to include work on all primary and secondary agricultural residues from which energy is being recovered; this includes biomass grown as an energy crop, i.e., crop-grown biomass. Another major aspect of this series is with respect to product from the bioconversion of biomass. The product from energy recovery is expanded in this present series to include gaseous fuel, liquid fuel, and organic chemicals. Thus, the intention is to include work on all aspects of biomass/bioconversion. Specifically, the projects and work described in this series are those dealing with cellulose, lignin, and fermentable industrial residues with the goal of the programs discussed being energy recovery in the form of gaseous fuel (methane), liquid fuel (most often, alcohol), and organic chemicals.

In addition to representing almost all of the work in the biomass/bioconversion solar energy area sponsored by the U.S. Department of Energy, this series has an international authorship of chapters. These volumes include a broad range of current work ranging from fundamental analysis and research work at the laboratory bench through discussion of engineering pilot plants and field demonstration units. Most of the work reported, due to the overall status of this emerging field, is seen to be strongly dedicated to what is appropriately termed development. In all cases these contributed chapters are designed to be largely tutorial in nature with the objective of providing the reader with an overall sense of direction, rather than being alternatives to publications in standard archival journals. It is to be noted that the editor requested from the experts working in this area to contribute chapters on their own work by describing in readable fashion the overall objectives, status, and especially the impact of their work. To the end of providing further insight into the socioeconomic impact of the potential results of all this biomass/bioconversion work, chapters have been included on technological assessment and economic analysis. On this basis, this series is intended to provide a reference and planning guide for the many industrial executives, plant managers, and state and local government officials who are now or certainly will be dealing with the demands of energy recovery. With this background, it will be useful to discuss the major areas included in this series on Bioenergy Systems.

In this series primary agricultural residues are defined as the waste material remaining following harvesting of grain, principally straw, as well as forest residues. Discussion of the location, amounts, and developmental programs underway for energy recovery from these primary residues form the basis for a major portion of this series. On the other hand, secondary agricultural residues are defined herein as that waste material remaining after some formal processing of an agricultural crop has taken place. On this basis, municipal solid waste may be considered as combined secondary agricultural residues. However, a less complex, single component secondary agricultural residue such as whey may be used as an example for discussion. Whey is the secondary agricultural residue that remains after the processing of milk to cheese. Continuing with this example, it may be shown that the whey from a typical modern cheese processing plant, if bioconverted to a gaseous or liquid fuel, may provide replacement of a substantial portion of the cheese plant's present petroleum derived fuel oil requirements.

In a similar manner, as with primary and secondary agricultural residues, bioconversion from a crop grown specifically for energy recovery is considered throughout this series. Often the term biomass is used to imply only crops grown solely for energy conversion, while primary and secondary agricultural residues are placed in a separate category termed, simply, wastes. People working in the area, however, generally classify all fermentable organic substrates together as biomass — recognizing the broad solar energy conversion aspects of all fermentable organic substrates, and this general classification is implied throughout the series. It is to be further noted at this point, with respect to bioconversion processing, that the specific objective is optimum production of a product. For many years the bioconversion field has been oriented to waste stabilization — only recently has attention been directed towards optimizing fuel production and the larger solar energy impact of this area been realized. Thus, the work included in the series is devoted to economic production of some valued product and does not simply include waste disposal. Clearly, commercial systems for any gaseous fuel, liquid fuel, or organic chemical production from biomass will emerge only if the energy balance and costs are economically competitive.

In addition to bioconversion to both gaseous and liquid fuels, the historical background, the basic fundamentals, the present status of development, and the potential for organic chemical production from biomass are also presented. In these portions of the series, contributed chapters depart from conventional methane and alcohol fermentations and deal with production of higher valued organic chemicals. These portions of the series provide the reader with insight into this rapidly emerging new field. It is interesting to note that prior to the development of the petrochemical industry an array of organic chemicals were made via fermentation routes. Moreover, selected fermentable feedstocks, including industrial residues, were often used for bioconversion to organic chemicals — some of these feedstocks are now being regarded again.

In many chapters dealing with gaseous fuel, liquid fuels, and organic chemicals an excellent review is provided of the fundamentals of these bioconversions and biomass. Also presented are the basic principles of the thermodynamics involved and the biochemical pathways understood as basic to product formation. Contributed chapters from workers in the field provide a ready insight to those interested in moving into this field or in capitalizing on developmental results. Also included are discussions on novel fermentations, chemical production by suppressing standard methane fermentation, and a number of other novel endeavors. Current progress on more classical fermentations such as acetone/butanol is also included. The broad question of longer range potential and the application of genetic engineering to produce organic chemicals are addressed. With this introduction, the organization of the series is reviewed, as well as the specific contents of each volume.

This series on Bioenergy Systems has been organized into five major volumes. *Fuel Gas Systems* and *Fuel Gas Developments* deal with fuel gas production from biomass, *Liquid Fuel Systems* and *Liquid Fuel Developments* deal with liquid fuel production from biomass, while *Bioconversion Systems* deals with unconventional processes, organic chemical production from biomass, and a broad overview of the impact of bioenergy systems in lesser developed countries.

Fuel Gas Systems and *Fuel Gas Developments* include chapters on both the research and development aspects of fuel gas production via anaerobic digestion. Specifically, *Fuel Gas Systems* tends to include the more practical or developmental aspects of fuel gas production from biomass — chapters deal largely with systems and engineering evaluations of total systems. *Fuel Gas Developments* includes a greater emphasis on current research and engineering topics or programs under development. It is to be noted that the first chapter in *Fuel Gas Systems* is by Dr. Roscoe F. Ward, now with the United Nations, but earlier responsible for carrying out the mission of the U.S. Department of Energy in the production of gaseous fuels, liquid fuels, and organic chemicals from biomass as Branch Chief of Fuels from Biomass. *Fuel Gas Developments* appropriately leads off with a chapter by Professor David A. Stafford, University College, Cardiff, Wales, U.K., who is well known for having initiated the International Symposia on Anaerobic Digestion. Both Dr. Ward and Dr. Stafford appropriately deserve having the lead chapters in each of these two volumes on fuel gas production from biomass.

As noted, *Fuel Gas Systems* and *Fuel Gas Developments* are devoted to gaseous fuel production via anaerobic fermentation. Excellent fundamental chapters are included in both volumes, as well as examples of practical research and development programs around the world. To single out for comment any one of these chapters in *Fuel Gas Systems* and *Fuel Gas Developments* dealing with fuel gas production from biomass would not be fair to any of the others. It is appropriate to point out, however, that the team leaders responsible for the experimental research and development work presented here are believed to be the foremost workers in the field of methane fermentation in the world. Each of these chapters represents more than a simple presentation of one research and/or development project — they each represent a continued and devoted effort to advance an entire field. Concluding chapters in each of these two volumes are given on the economic feasibility for fuel gas production from agricultural residues of various types. These economic analyses and technical assessments from authors at Dynatech were carried out for the Solar Energy Research Institute (SERI) of the U.S. Department of Energy. It is appropriate here to thank Mr. Dan Jantzen, now with the U.S. Agency for International Development, Katmandu, Nepal, who was Program Manager at SERI and responsible for coordinating projects in the area of fuel gas production from biomass via anaerobic fermentation.

Included in *Fuel Gas Systems* is a two-part assessment of secondary agricultural residues, indicating the broad array of potential sources for initiating energy recovery systems. In keeping with environmental concerns about removal of primary crop residues from the land for purposes of energy recovery, an assessment of this potential problem is also included. To conclude *Fuel Gas Developments* on a practical and economic note, and in keeping with the theme of this entire series, a comparative study of converting agricultural crop residues to either gaseous or liquid fuels is presented.

Liquid Fuel Systems and *Liquid Fuel Developments* are devoted to liquid fuel production. Here the organization of *Liquid Fuel Systems,* is to place together chapters on complete systems, i.e., processes under development or undergoing economic assessment. The organization of *Liquid Fuel Developments,* is to include those chapters dealing with more specific research problems. On the other hand, these are really two complete volumes on liquid fuel production from biomass. It is meaningful to note that chapters in these two volumes are largely from groups funded by the U.S. Department of Energy, where the major effort on alcohol from cellulose is being conducted. One novel project on alkane fuel from biomass is also described. The chapters on experimental alcohol fuel development include basic work at the University of Georgia, Rutgers University, U.S. Army Natick R&D Laboratory, University of Connecticut, and General Electric. More process development oriented research is presented from Lehigh University (the workers most recently at the University of Pennsylvania), Dartmouth College, Georgia Institute of Technology, University of Arkansas, and New York University. Because the effort on alcohol fuel from cellulose has had major U.S. Department of Energy funding, it is seen that most of the chapters describing experimental programs are from workers in the U.S. On the other hand, work in Brazil and Scotland is presented. It is also interesting to note that the technical assessments and economic evaluations included have been carried out with a world-wide perspective.

It is meaningful to note that the lead chapter in *Liquid Fuel Systems,* is by Prof. H. R. Bungay, now with Rensselaer Polytechnic Institute who was responsible for initially organizing the U.S. Department of Energy programs in the area of liquid fuels and organic chemicals from biomass.

Bioconversion Systems is devoted to unconventional systems, production of organic chemicals from biomass, and a perspective of bioenergy systems to lesser developed countries. Perhaps because of the newness of this area of investigation, the chapters range from a very thought-provoking one on the potential for genetic engineering, to one as practical as the conversion of lignin to useful chemical products. Several chapters on chemicals from a variety of biomass feedstocks are included as is a very useful economic assessment. A culminating chapter to this entire series is on integrating a crop-grown biomass system with an on-farm anaerobic digestion system. This chapter is presented to show how the future of fuels and organic chemicals may evolve. Finally, to provide a broad evaluation of the impact of biomass to liquid fuel in developing countries, a comprehensive series of four chapters is presented.

Hopefully, all the work described in this series in Bioenergy Systems will inspire those now working in the field and will encourage those who are now beginning to investigate this field.

Collecting and organizing the work reported in this series has been a very meaningful experience for the editor. Especially heartwarming have been the working with old friends and meeting with new friends. The detailed work of organizing and corresponding with sometimes tardy authors, the helpful and time consuming work of making the chapters as readable as possible, and the communication with our publisher have been done by Joyce E. Perkins, Ph.D. Dr. Perkins also brought a joy to her work that made her sometimes tedious tasks more pleasant and in this manner assisted the editor immeasureably. We are all very appreciative of her expertise as an English scholar and her executive talents.

Donald L. Wise, Ph.D.
Editor

PREFACE

With the emphasis in this volume on individual developments with liquid fuel, rather than total systems, an excellent research review of fundamental aspects of ethanol from biomass is by Carreira and Ljungdahl, from the University of Georgia. Following this are chapters from some of the leading workers in this newly emerging field and representing the major commitment of the U. S. Department of Energy funds in this area. These chapers are by Eveleigh of Rutgers, Allen of U. S. Army Natick (where the concept of enzymatic hydrolysis of cellulase to glucose and subsequently to alcohol was initiated), Phillips and Humphrey of Lehigh (most recently at the University of Pennsylvania), Grethlein and Converse of Dartmouth, Colcord and Bery of Georgia Tech, and Rugg and his co-authors of New York University. A novel approach to liquid fuel production via suppressed methane fermentation and sponsored by the Solar Energy Research Institute is given by Levy and his co-authors of Dynatech. Concluding this volume is a very thought-provoking economic evaluation of producing chemicals and fuels from agricultural crops by Wagner and his co-authors of Battelle Memorial Institute. Together *Liquid Fuel Systems* and *Liquid Fuel Developments* both include all major aspects of liquid fuel from biomass.

THE EDITOR

Donald L. Wise, Ph.D., is Vice President of Dynatech R/D Company, Cambridge, Mass. Dr. Wise received his B.S. (magna cum laude), M.S., and Ph.D. degrees in chemical engineering at the University of Pittsburgh. Dr. Wise is a specialist in process and biochemical engineering as well as advanced biomaterials development. At Dynatech he has managed a series of programs to develop processes for production of fuel gas, liquid fuels, and organic chemicals from municipal solid waste, an array of agricultural residues, and a wide variety of crop-grown biomass, especially aquatic biomass. Dr. Wise has also been primarily responsible for the initiation of development work on peat to gaseous fuel, liquid fuels, and organic chemicals, and he also originated work on the bioconversion of coal gasifier product gases to these products. This work of Dr. Wise has been carried out from laboratory experiments to pilot-scale operation to full-scale demonstration.

Dr. Wise has worked in the area of biotechnology research and development for two decades and has approximately fifty publications in the field. As Associate Editor of *Solar Energy,* the journal of the International Solar Energy Society, he is responsible for the review of manuscripts in the biomass/bioconversion area. Dr. Wise is also on the Editorial Board of *Resources and Conservation,* an international journal published by Elsevier, Amsterdam. He is an officer of the Biotechnology and Chemicals Sciences Division of the American Society for Solar Energy, a section of the International Solar Energy Society.

Recently Dr. Wise initiated a program to establish the engineering feasibility of converting large-scale combined agricultural residues to fuel gas by the action of microorganisms. Earlier, feasibility study work directed by Dr. Wise on the ''controlled landfilling'' of municipal solid waste for enhanced methane recovery led to several demonstration projects at landfill sites around the U.S. He initiated the program at Dynatech on organic chemical production from biomass sponsored by the U.S. Department of Energy. Dr. Wise also initiated a unique program for syngas fermentation, i.e., the fermentation of CO, CO_2, and H_2 to methane and also organic chemicals. Dr. Wise was also responsible for an economic assessment of fuel gas production from animal residues for the U.S. Department of Energy and earlier coordinated the work in this area. The development of a peat biogasification process was initiated by Dr. Wise. He has also probed the economics and impact of large-scale aquatic biomass farming.

Prior to this work, Dr. Wise initiated a program at Dynatech for the production of fuel gas from solid waste by anaerobic digestion. The experimental laboratory feasibility study was carried out by Dr. Wise. Continuing with the success of this experimental program, Dr. Wise organized a broad interdisciplinary team to carry out the computer-based economic analysis of a 1000 ton/day plant servicing a population of 400,000 for bioconversion of solid waste. Dr. Wise has supervised a pilot plant for the conversion of up to 100 lb/day of solid waste to fuel gas.

At the present time, Dr. Wise is involved in the production of higher valued products from the bioconversion of biomass including the production of pentanol, a high octane booster for gasoline, as well as selected organic chemicals. He is also currently working on the utilization of biomass lignin with the objective of developing a commercial process for production of a BTX type liquid fuel.

A meaningful portion of these programs that Dr. Wise initiated and has been carrying out is his meeting with experts across the U.S. and around the world to become familiar with both current and practical aspects of fuels and organic chemicals from biomass.

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Chapter 1

PRODUCTION OF ETHANOL FROM BIOMASS USING ANAEROBIC THERMOPHILIC BACTERIA

Laura H. Carreira and Lars G. Ljungdahl

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I. INTRODUCTION

A. Scope

The industrialized society of today is strongly dependent on liquid fuels such as oil and gasoline as a source of energy for chemical feedstocks. These fuels are supplied by some few countries which to a large extent have controlled the world's economy during the last decade. In addition our consumption of these fuels is high and we will within this century deplete a large part of our nonrenewable resources of liquid fuels. For these reasons efforts are being made to partially substitute ethanol for gasoline and oil. Ethanol can be produced by fermentation of carbohydrates from renewable resources (biomass).

Ethanol fermentation of sugars and of hydrolyzed starch by yeast is one of the oldest biological processes known to man and it is the basis for the brewing industry. However, extensive research is being conducted to find new fermentative processes for ethanol production. These processes will be based on cellulosic and hemicellulosic biomass as well as on starch rather than simple sugars and hydrolyzed starch.

To convert biomass to ethanol and other organic feedstock chemicals using biological methods research is now conducted in at least five different areas: (A) evaluation of available biomass resources such as residues from forestry and agriculture and of municipal waste and the development of energy plantations; (B) pretreatment of cellulosic material including removal of lignin and hydrolytic procedures; (C) evaluation of known and newly isolated microorganisms to be used in fermentations of cellulosic material, starch or other carbohydrates and the use of mutation or other genetic techniques to obtain more efficient strains; (D) improved fermentation technology including fermentations using stationary or immobilized cells and in two-phase systems; (E) methods for recovery of fermentation products such as vacuum distillation, membrane filtration, phase partitioning, and adsorption. The main intention of this paper is to cover (C) and then almost exclusively the use of anaerobic thermophilic microorganisms for the production of ethanol from biomass. Other areas will only be casually covered.

B. Why Thermophilic Microorganisms?

Thermophilic microorganisms grow optimally at temperatures above 40°C. The extreme thermophiles grow over 70°C and some of them are able to live in boiling water.[1,2] Extensive recent reviews of properties and the physiology of thermophiles have been published.[3-7] Fermentations of biomass using bacteria are dealt with in several new papers. Wiegel[8,9] has pledged for the use of anaerobic thermophilic bacteria in industrial ethanol fermentations. Avgerinos and Wang[10] have discussed the direct microbiological conversion of cellulosics to ethanol. Zeikus[11,12] has reviewed chemical and fuel production using thermophilic bacteria, Rosenberg[13] has discussed fermentations of pentose sugars to ethanol and other products, Volesky et al.[14] has critically reviewed acetone-butanol-ethanol fermentations, and several authors have covered related topics in the book *Trends in the Biology of Fermentations for Fuels and Chemicals.*[15]

1. Features of Fermentation with Yeast

The fermentation of glucose to ethanol as carried out by yeast (generally *Saccharomyces cerevisiae*) has been practiced for thousands of years. The process is well developed, relatively simple, efficient, and the yield of ethanol is high. However, *S. cerevisiae* ferments with good rates only glucose, maltose, sucrose, and fructose and is thus unable to ferment xylose and the polysaccharides. The latter constitute the main proportion of the biomass. Hydrolysis either enzymatically or chemically of starch and cellulose yields glucose which can be fermented by yeast. Simultaneous hydrolysis of cellulose in the presence of the cellulase enzyme complex from *Trichoderma reesei* and whole cells of *S. cerevisiae* to ethanol has been successfully attempted.[15] However, xylose the product of hydrolysis of hemicellulose, is not fermented by *S. cerevisiae,* but it can be utilized by other yeast species in secondary fermentations[17-19] or by bacteria.[13] An interesting approach is the fermentation of xylose to ethanol in the presence of xylose isomerase and yeast.[20,21] The xylose isomerase converts the xylose to xylulose, which is metabolized by several yeasts.

The yeast normally used in ethanol fermentations is quite sensitive to temperature and ferments optimally around 30°C and maximally at 39°. This means that pretreated or sterilized substrate must be cooled before inoculation and that cooling may have to be applied during the fermentations. This is difficult or expensive especially in countries with warm climates. Yeast fermentations are also sensitive to the pH, which for optimum fermentations must be narrowly controlled around 4.2.

2. *Features of Fermentation with Zymomonas*

A second type of fermentations, which is used to produce alcoholic beverages, is with bacteria of the genus *Zymomonas.* An excellent review of the biology of this genus has been written by Swings and De Ley.[22] Fermentations with strains of *Zymomonas,* like yeast, yield 2 mol of ethanol and 2 mol of CO_2/mol of glucose. However, the fermentative pathway in *Zymomonas* is according to the Entner-Doudoroff mechanisms,[23] whereas in yeast it is by the Embden-Meyerhof pathway.

Recently, a substantial interest has developed for the use of *Zymomonas* strains in the production of industrial ethanol. The group of Rogers[24-26] in Australia has evaluated the rate of growth and ethanol production by 11 strains of *Zymomonas.* They studied the effect of temperature on the fermentation and shown that *Z. mobilis* (ATCC 10988) have higher rates of sugar uptake and ethanol production than the traditional yeast fermentation. With a continuous cell recycling system they reported[24] an ethanol production from glucose of 120 g ℓ^{-1} h^{-1}. Unfortunately, *Zymomonas* strains grow only on fructose and glucose and some strains after apparent induction also on sucrose.[22] Thus the range of sugars fermented by *Zymomonas* is similar to that of yeast. The optimum temperature for fermentations with *Zymomonas* is about 30°C although it will ferment up to 40°C but with a lesser yield.[25] The pH range is between 4 and 7.5.[22] Some strains of *Zymomonas* when growing on sucrose produce levan,[22-26] which is a polymer of fructose with a molecular weight of about 10^7. Levan formation, if it occurs, clearly decreases the yield of ethanol. It may also interfere with determinations of growth rates using optical density.

Although fermentations using *Z. mobilis* appear favorable in comparison with yeast fermentations, they exhibit the same limitations as the latter. Therefore, search for other more versatile microorganisms is warranted. Of particular value would be the discovery of microorganisms that are capable of directly and efficiently fermenting starch, cellulose and hemicellulose.

3. *Overview of Types of Anaerobic Thermophilic Bacteria*

A large proportion of organic material including cellulose and hemicellulose, which accumulate by photosynthetic fixation of carbon dioxide in nature, is converted to methane and carbon dioxide by anaerobic bacteria.[27,28]

This is shown for cellulose in Reaction 1.

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow n(3CH_4 + 3CO_2) \qquad (1)$$

This process is complex and involves a consortium of several bacterial types that interact.[29-32] In Figure 1, which is a simplified diagram, it is seen that Reaction 1 is a composite

A. PRIMARY BACTERIA

Cellulose $\longrightarrow$ cellobiose + glucose $\longrightarrow$ lactate + ethanol + acetate + CO_2 + H_2

B. SECONDARY BACTERIA

1. cellobiose + glucose $\longrightarrow$ lactate + ethanol + acetate + CO_2 + H_2
2. cellobiose + glucose $\longrightarrow$ ethanol + CO_2
3. glucose $\longrightarrow$ acetate

C. ANCILLARY BACTERIA

1. lactate $\longrightarrow$ acetate + CO_2 + H_2
2. ethanol $\longrightarrow$ acetate + H_2

D. METHANOGENIC BACTERIA

1. acetate $\rightarrow CO_2$ + CH_4
2. $CO_2 + H_2 \rightarrow CH_4$

FIGURE 1. Bacterial types required for the complete anaerobic fermentation of cellulose to methane and carbon dioxide.

of the action of at least four groups of bacterial types. The primary bacteria, Group A, hydrolyze cellulose to oligo-saccharides, cellobiose and glucose and also ferment these sugars to lactate, ethanol, acetate, carbon dioxide, and hydrogen. The secondary bacteria, Group B, ferment di- and monosaccharides to lactate, ethanol, acetate, carbon dioxide, and hydrogen. The secondary bacteria can be divided up in sub-groups, of which three are shown in Figure 2. Group B1 yields a mixture of products, Group B2 forms ethanol and CO_2, and Group B3 are homoacetate fermenting bacteria. Many of the bacteria of Group B3 grow autotrophically and are able to synthesize acetate from CO_2 and H_2, methanol and carbon monoxide. This group will be discussed in a separate chapter of this book.[33] The ancillary bacteria, Group C, convert lactate to acetate and hydrogen. They also convert ethanol to acetate and hydrogen. Sulfate reducing and similar bacteria which reduce inorganic compounds with electrons from organic substrates belong to this group.[34] The methanogenic bacteria, Group D, split acetate into methane and carbon dioxide and synthesize methane by the reduction of carbon dioxide with hydrogen.[27,29]

The fermentation of cellulose to methane and carbon dioxide occurs in an anaerobic thermophilic environment. Anaerobic thermophilic bacteria occur also in a ''normal'' environment and they can be isolated from mud or soil samples from fields, rivers, and lake banks, from composts of straw, bark, household and garden refuse, and from horse manure.[35] Wiegel and Ljungdahl[29] have isolated from a single soil sample collected in Athens, Georgia all anaerobic thermophilic bacteria necessary to complete the reactions shown in Figure 1. Table 1 is a list of anaerobic thermophilic bacteria, which carry out these reactions.

In this paper we will discuss the primary bacterium, *Clostridium thermocellum,* and the secondary bacteria of sub-groups 1 and 2. All these bacteria produce ethanol from carbo-