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BIOGAS
PRODUCTION & UTILIZATION

By
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PREFACE

Increasing energy costs have led to an interest in the value and recovery potential of waste materials. Development of methods used in waste disposal and energy recovery schemes is under continuing examination. Greater degrees of technology, chemistry and biology are being focused on wastes for materials and energy recovery as opposed to traditional methods of disposal. Decomposition of organic wastes can produce gases whose major constituent is methane, a primary component of natural gas, a fuel of high energy and economic value.

The purpose of this book was to establish an overview for recovery processes which ultimately depend on biological activity. The use of biological systems in solid waste treatment has not in general been employed for resource recovery purposes because of the historical availability of cheap energy from other sources. However, biological treatment can be simultaneously a waste disposal and resource recovery system. While the use of such systems is not new the growing costs of energy magnifies the interest and potentials. It is within this context that we consider the biological conversion process, specifically anaerobic digestion and some of its applications.

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CONTENTS

CHAPTER 1

MICROBIOLOGY AND BIOCHEMISTRY

The microbiological degradation of organic material in an anaerobic environment can only be accomplished by microorganisms able to utilize molecules other than oxygen as hydrogen acceptors. This anaerobic decomposition ultimately results in the production of a biogas consisting of methane (50–70%), carbon dioxide (25–45%) and small amounts of hydrogen, nitrogen and hydrogen sulfide. The overall chemical reaction is often simplified to:

$$\text{organic matter} \xrightarrow[\text{microorganisms}]{\text{anaerobic}} CH_4 + CO_2 + H_2 + N_2 + H_2S$$

In fact, the anaerobic degradation of organic material is, chemically, a very complicated process involving hundreds of possible intermediate compounds and reactions, each of which is catalyzed by specific enzymes or catalysts. Many of the transformations may be accomplished by one of several alternative metabolic pathways, and the biochemists continue to attempt to define and describe more precisely the various mechanisms.

The ability of an organism to effect any particular transformation or reaction depends on the availability of the enzyme or catalyst specific to that reaction, and in the case of intercellular reactions, the ability of the substrate to pass through the cytoplasmic membrane of the cell.

Anaerobic decomposition is generally considered to progress in two stages: an acid production stage and a methane production stage.

ACID PRODUCTION STAGE

The first step in the acid production stage is the hydrolysis and liquefaction of the large, insoluble organic molecules by extracellular enzymes.

The carbohydrases catalyze the hydrolysis of glycosidic bonds. Starch and glycogen, for example, are hydrolyzed to a disaccharide by the action of one of a group of enzymes called amaylases. These enzymes attack polysaccharides from the nonreducing end of the chain cleaving alternate glycosidic bonds as shown in Figure 1. The disaccharides are then cleaved to monosaccharides by a glycosidase. The specific enzyme involved depends on the nature of the glycosidic bond and the monosaccharide involved; that is, whether the configuration is alpha or beta, dextro or levo, and the size of the heterocyclic ring. Cellulase and chitinase degrade the structural polysaccharides cellulose and chitin.

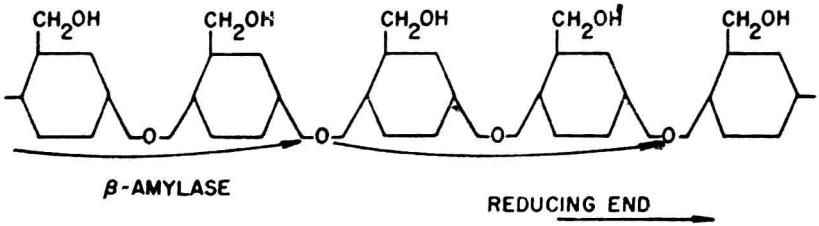

Figure 1. Cleavage of alternate glycosidic bonds by B-amylase.

Lipases and esterases hydrolyze fats and lipids. When the substrate is a mixed triglyceride of long-chain fatty acids similar to those found in natural fats or oils, the hydrolysis proceeds in a stepwise manner, with the rapid formation of di- and monoglycerides, followed by slow hydrolysis of the monoglycerides.

Proteolytic enzymes, proteases, catalyze the hydrolytic cleavage of the peptide bonds of proteins. Again, these enzymes are somewhat specific. Exopeptidases are restricted to terminal peptide bonds but endopeptidases must attack peptide bonds that are centrally located in the peptide chains. The enzyme trypsin is specific for bonds involving the amino acids argenine or lysine [1].

After this hydrolysis and liquefaction, these initial degradation products are in a form that can be utilized by the microorganisms, providing they are able to pass into the cell through the cytoplasmic membrane. This membrane selectively regulates the flux of nutrients, ions and waste products in and out of the cell. It is composed of lipids and proteins. The lipids provide the structural properties, and the proteins provide the membrane with its distinctive functional properties. It is believed that the various proteins embedded in the membrane act as revolving

gates for specific substances. This explains the ability of the membrane to pump against the head of an osmotic pressure gradient, a phenomena called active transport. The lactose-transport system of *E. coli,* for example, is capable of producing a lactose concentration 500 times greater inside the cell than outside. The rotation of these protein gates requires a certain amount of membrane fluidity, which is largely determined by the relative amounts of unsaturated and saturated fatty acids in the lipid portion. Bacteria grown at a low temperature have membranes with a greater proportion of unsaturated fatty acid than those grown at a higher temperature [2].

A typical metabolic pathway for the further degradation of carbohydrates is the Embden-Meyerhof-Parnas pathway of glycolysis as shown in Figure 2 [3]. Note that the end product of glycolysis in yeast is ethanol, and in bacteria is acetic acid.

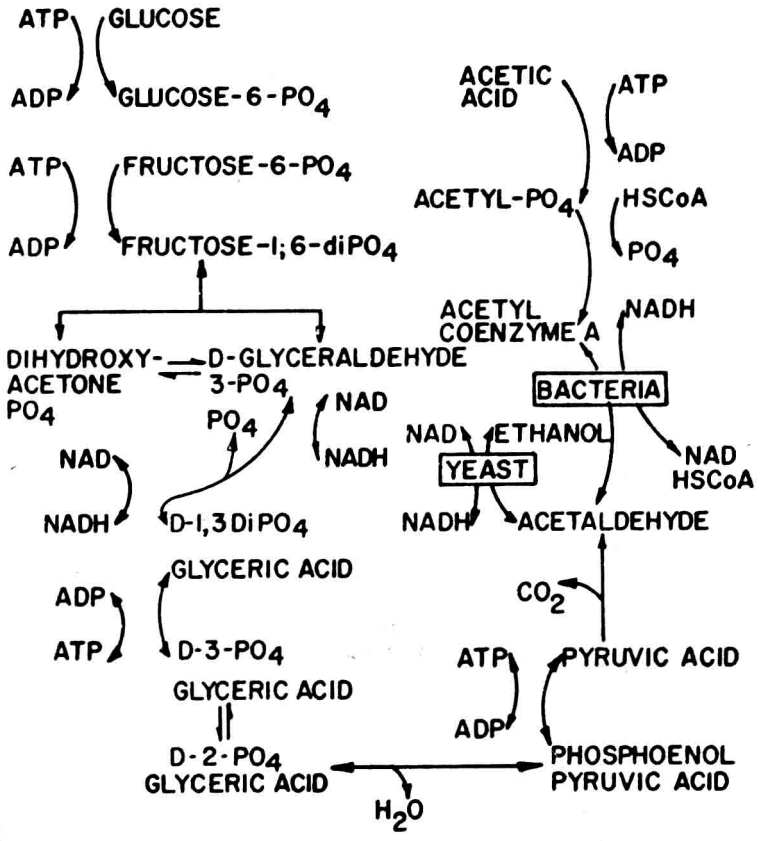

Figure 2. Embden-Meyerhof-Parnas pathway of glycolysis.

Carbon dioxide is evolved in either case. ADP and ATP represent adenosine diphosphate and triphosphate, respectively. Energy from exothermic transformations is stored in high-energy phosphate bonds of ATP which may be regarded as a charged storage battery. This energy may be released to drive endothermic reactions, at the same time transforming the ATP to ADP. Nicotinamide-adenine dinucleotide (NAD) is a coenzyme often involved in hydrogen transfer reactions, which must be regenerated with a coupled reaction.

In recent years, carbon 14 tracers have been used in substrates to aid in definition of the metabolic pathways. It is generally agreed [3-5] that the anaerobic degradation of long-chain fatty acids proceeds primarily via beta oxidation, in which two carbon atoms at a time are split from the chain. The sequence in Figure 3 is shown for the removal of one acetate unit which combines with reduced coenzyme A (CoASH).

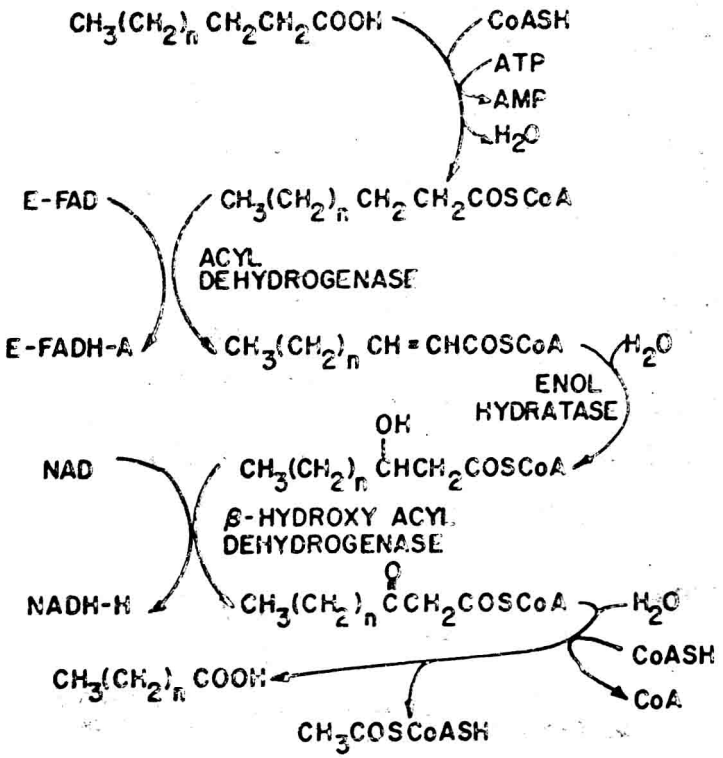

Figure 3. Beta oxidation of fatty acids.

Acetic acid can then either be liberated from CoA in a subsequent reaction or the acetate can be transferred to other functional compounds. Flavin adenine dinucleotide (FAD) is a prosthetic group for a wide variety of enzymes (E-FAD) associated with hydrogen transfer reactions.

Amino acids can be biologically dissimilated by any of several pathways, depending on the enzyme system available. From their studies using carbon 14 tracers during the anaerobic digestion of glutamic acid, Weng and Jeris [5] have postulated the mechanism shown in Figure 4.

Some of the species of bacteria which participate in the acid production stage and have been isolated from anaerobic digesters are listed in Table 1 [6].

METHANE PRODUCTION STAGE

The low-molecular-weight acids produced in the acid production stage are further degraded to methane and carbon dioxide by a highly specialized group of bacteria commonly referred to as the methane producing bacteria. These

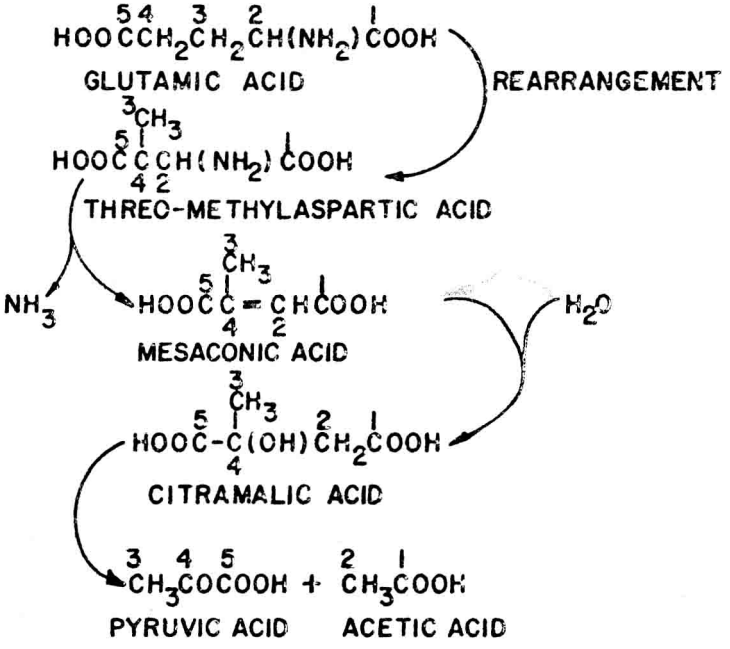

Figure 4. Degradation of glutamic acid.

Table I. Nonmethanogenic Bacteria Isolated from Anaerobic Digesters [6]

Bacterium \ Isolated on	Cellulose	Starch	Protein		Lipid
			Peptone	Casein	
Aerobacter aerogenes					
Alcaligenes bookerii					X
A. faecalis	X				
Bacillus sp					
B. cereus var. *mycoides*		X		X	
B. cereus	X	X	X	X	
B. circulans			X		
B. firmus			X		
B. knelfelhumpi					
B. megaterium	X	X		X	X
B. pumilis			X	X	
B. sphaericus			X	X	X
B. subtilis			X	X	X
Clostriduim carnofoetidum	X				
Escherichia coli			X	X	
E. intermedia					
Micrococcus candidus		X			
M. luteus					X
M. varians		X	X	X	
M. ureae		X			
Paracolobacterium intermedium			X		
P. coliforme			X		
Proteus vulgaris	X				
Pseudomonas aeruginosa	X				
P. ambigua					
P. oleovorans					X
P. perolens					X
P. pseudomallei					
P. reptilivora	X				
P. riboflavina	X				X
P. spp.	X	X	X	X	X
Sarcina cooksonii					
Streptomyces bikiniensis					X

organisms have the unique ability to couple organic oxidation to reduction of carbon dioxide. In this process carbon dioxide is the terminal hydrogen acceptor and is analogous to oxygen in aerobic respiration.

Four genera of strictly anaerobic bacteria are known to produce methane:

1. *Methanobacterium*, a nonspore-forming rod;
2. *Methanobacillus*, a spore-forming rod;
3. *Methanococcus*, a nonspore-forming coccus; and
4. *Methanosarcina*, a nonspore-forming coccus in packets of eight.

Each species within the four genera is quite restrictive with regard to the organic acid or alcohol it can use as a carbon source. Oxidation of the substrate by a single species is often incomplete, and the partial degradation product can act as a substrate for another species. Table II lists some of the species and the organic compounds they can use.

Table II. Methanogenic Bacteria [6]

Bacterium	Substrates	Products
Methanobacterium formicum	CO	CH_4
	H_2+CO_2	
	Formate	
M. mobilis	H_2 + CO_2	CH_4
	Formate	
M. propionicum	Propionate	CO_2 + acetate[a]
M. ruminantium	Formate	CH_4
	H_2 + CO_2	
M. sohngenii	Acetate butyrate	CH_4 + CO_2
M. suboxydans	Caproate and butyrate	Propionate and acetate[a]
Methanococcus mazei	Acetate and butyrate	CH_4 + CO_2
M. vannielii	H_2 + CO_2	CH_4
	Formate	
Methanosarcina barkeri	H_2 + CO_2	CH_4
	Menthanol	CH_4
	Acetate	CH_4 + CO_2
M. methanica	Acetate	CH_4 + CO_2
	Butyrate	

[a]Acetate or propionate converted to Ch_4 in a two-step process.

Two mechanisms have been postulated for the conversion of acetate to carbon dioxide and methane. Van Niel published his Carbon Reduction Theory in 1938 [7]. The basis of this theory is that since the nature of the end products is independent of the original substrate structure, the compounds are initially oxidized to carbon dioxide, followed by a reduction process in which some or all of the carbon dioxide is reduced to methane, or:

Oxidation $CH_3COOH + 2H_2O \longrightarrow 2CO_2 + 8H$

Reduction $8H + CO_2 \longrightarrow CH_4 + 2H_2O$

Net $CH_3COOH \longrightarrow CH_4 + CO_2$

Other work has confirmed the existence of this pathway.

More recent studies using carbon 14 and deuterated water have demonstrated an alternative pathway [8]:

$$C^{14}H_3COOH + D_2O \longrightarrow C^{14}H_3D + CO_2$$

then:

$$CO_2 + 8H^+ \longrightarrow CH_4 + 2H_2O$$

which indicates that methyl groups may go directly to methane without the carbon dioxide intermediate step.

The general biochemical sequences for the degradation of organic compounds are summarized in Figure 5. Pyruvic and acetic acids occupy key intermediate positions from which further biochemical reactions originate. They may serve as building blocks for the synthesis of more complicated organic molecules, be converted to the intracellular storage product poly-beta-hydroxybutyric acid (PBH), or be further degraded to ethanol by yeast or to methane and carbon dioxide by the methane bacteria.

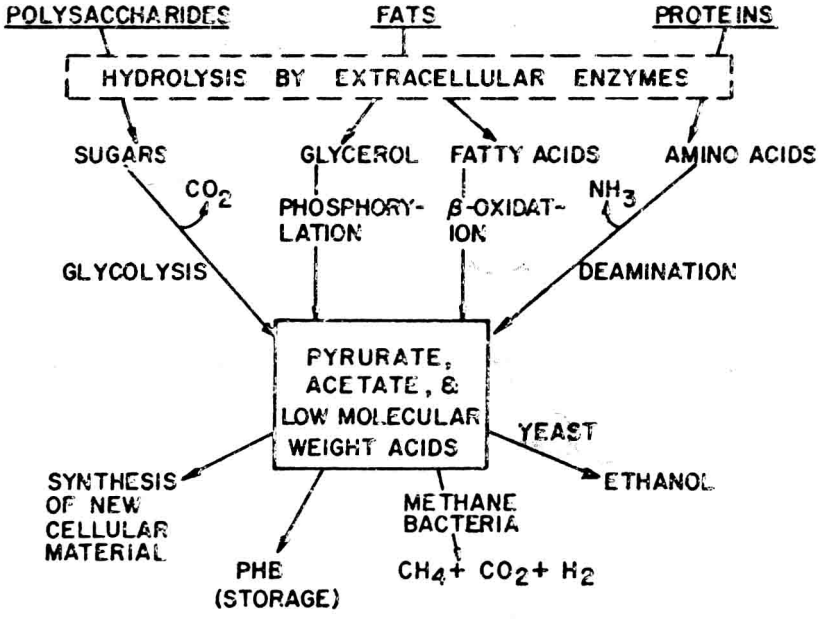

Figure 5. Principal pathways of degradation of organic compounds.

In the absence of oxygen, some heterotrophic bacteria utilize the sulphur and nitrogen of the sulfate and nitrate anions as electron acceptors. (Another way of looking at the process might be to say that oxygen from the sulfate and nitrate anions is used as a hydrogen acceptor.) The bacterium Desulfovibro, a strictly anaerobic, Gram-negative, nonsporeforming rod, grows heterotrophically on many different organic substrates and can use sulfate, thiosulfate or elemental sulfur as electron acceptors. Under acidic conditions the sulfide radical formed will be converted to hydrogen sulfide, a toxic gas which in small concentrations can cause odor problems and/or contribute to corrosion of iron pipes and crown corrosion of sewer pipes.

The process of the bacteriological conversion of nitrates to nitrogen is called denitrification, and it proceeds in the following steps [9].

Nitrate		Nitrite		Nitrous oxide		Nitrogen
NO_3^-	$\longrightarrow$	NO_2^-	$\longrightarrow$	N_2O	$\longrightarrow$	N_2

The most common denitrifying bacteria are:

Bacillus denitrificans,
Micrococcus denitrificans,
Pseudomonas stutzeni and
Achromobacter,

although hundreds of strains have been isolated.

The reduction of nitrate acounts, in part, for the lack of fertility of constantly wet soils supporting growth of anaerobic species and, in part, for the function of swampy lands as a nutrient sink.

Recent studies with nitrogen 15 labeled $Ca(NO_3)_2$ have provided evidence that some of the nitrates are reduced to ammonium form [8].

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CHAPTER 2

FACTORS AFFECTING THE ANAEROBIC PROCESS

Widespread application of the anaerobic process has been hampered somewhat by its reputation as being easily upset and unreliable. Further development of anaerobic process technology is dependent on a better understanding of the factors associated with the stability of the biological processes involved. Process instability is usually indicated by a rapid increase in the concentration of volatile acids, with a concurrent decrease in methane gas production, indicating that the more fastidious methane bacteria are the most susceptible to upset.

Optimum conditions and ranges for anaerobic digestion have been studied by many investigators who are not always in agreement. One reason for this may be that their studies are conducted using different feed materials as well as different methodologies. The nature and composition of the substrate material dictate the microbial regime present, and it appears that a single set of parameters is not valid for all situations [1].

Many of the laboratory and pilot-scale studies reporting process failures have used acclimation periods of a month or less. Adaptation of the microorganisms to a substrate has been reported to take more than five weeks [2], and sufficiently acclimated bacteria have shown unusual stability toward stress-inducing events such as hydraulic overloads, fluctuations in temperature and in volatile acid and ammonia concentrations [3].

TEMPERATURE

Digestion Temperatures

Digestion and gas production can occur over a wide range of temperatures (4–60°C) if the temperature is held constant. Once an effective temperature range