Metabolism of glucose and acids in stressed anaerobic digestors : an investigation by 13C-NMR spectroscopy

by Elizabeth Ann Runquist

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Chemistry
Montana State University

Abstract:
The metabolism of glucose and the acids produced from glucose were studied in non-stressed and stressed anaerobic digesters. The utilization of glucose and the acids, acetate and propionate, were observed by 13C-nmr spectroscopy. Rate constants were obtained for both stressed and non-stressed fermentations. The rate constants obtained for propionate utilization suggested that propionate oxidation was a limiting factor in the anaerobic fermentation of glucose.

The observed carbon flow from 13C-l-glucose to the acids indicated that a major portion of the glucose was initially converted to lactate which was subsequently metabolized to either propionate or acetate. These studies confirmed an earlier suggestion that under high energy loads a major portion of the glucose was converted to reduced acids e.g, propionate. Under these conditions, these investigations indicated that approximately 70% of the glucose fed was converted to propionate. Thus, the methanogenic bacteria obtained a limited suppply of acetate rather than a saturating level. Possible metabolic intermediates of propionate catabolism indicated that both the acrylate and randomizing pathways had been utilized.

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Date 4 August 1981

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by

ELIZABETH ANN RUNQUIST

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Approved:

John E. Robbins
Chairperson, Graduate Committee

Edwin H. Abbott
Head, Major Department

Michael P. Malone
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

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ABSTRACT

The metabolism of glucose and the acids produced from glucose were studied in non-stressed and stressed anaerobic digestors. The utilization of glucose and the acids, acetate and propionate, were observed by ^{13}C-nmr spectroscopy. Rate constants were obtained for both stressed and non-stressed fermentations. The rate constants obtained for propionate utilization suggested that propionate oxidation was a limiting factor in the anaerobic fermentation of glucose.

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INTRODUCTION

Anaerobic Digestion - the Ecosystem

Today, with much emphasis being placed on the diminution of our energy resources, greater attention is being directed towards the exploration of alternative sources for energy. Anaerobic treatment of waste for the production of methane gas is one such alternative. McCarthy (45) has estimated that twenty percent of the natural gas consumed in the United States could be provided from the anaerobic treatment of municipal sludges and refuse, animal wastes and crop residues.

Since the nineteeth century the process of anaerobic digestion has been employed for the production of energy (40). To date, however, its widespread application has been limited by a lack of resolution of the optimum conditions for the production of methane. Kroeker (40) has suggested that the key to the feasibility of this alternative energy source lies in the understanding of the biochemical aspects of the process and their relationship to the stability of the system.

The production of methane via anaerobic digestion is accomplished through an ecosystem comprised of about twenty-five species of non-spore forming bacteria (67). These bacteria have been classified into three major groups (some authors have divided the microorganisms into only two groups (20,30,40) whereas some authors have referred to four groups present in the process (17)). Bryant (13) has classified the three major groups as the fermentative, acetogenic and methanogenic bacteria. The production of methane results from the interactions of these three groups of anaerobic bacteria.

The Fermentative Group

The first step in the anaerobic process involves a group of faculative anaerobic bacteria known as the acid formers or the fermentative group. During this stage of the process no methane is produced, however, the importance of this step lies in the process by which complex organic material is converted into usable substrates for the methanogens.

The functional role of the fermentative group in the overall anaerobic digestion process is the conversion of the polymeric substances into simple organic acids, alcohols, H_2O and CO_2 which can be utilized by the methanogens. Substrates which are hydrolyzed by this group include polysaccharides, lipids and proteins. The polymers can be converted into such compounds as butyrate, acetate, propionate, ethanol, H_2 and CO_2. A representative metabolic pathway is summarized in Figure 1. The polysaccharide is hydrolyzed to glucose which is then metabolized to pyruvate by the glycolytic pathway. The fate of pyruvate is determined by metabolic interactions of the entire anaerobic ecosystem. This will be elaborated upon in the section entitled Function of H_2: Ecosystem Interactions. Pyruvate may be converted, by the fermentative group, to acetate, CO_2, H_2, ethanol,

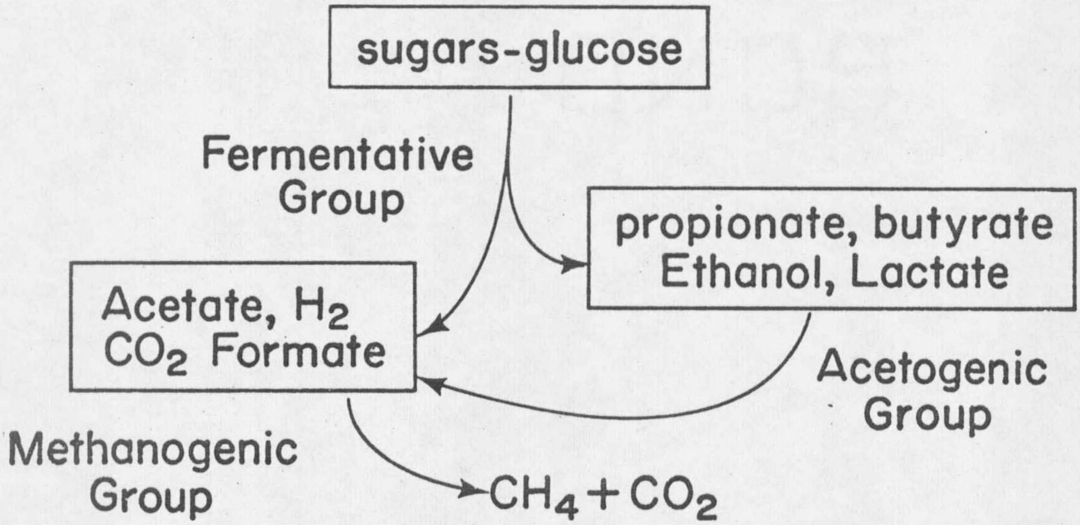

Figure 1. The three stages involved in the production of methane (13).

butyrate or propionate. The general metabolic scheme of the fermentative bacteria is shown in Figure 2.

The H_2-Producing-Acetogenic Bacteria

Some products of the fermentative process are not substates of the methanogenic group. There is a secondary process which must occur that utilizes the products, other than acetate, CO_2 or H_2. This process involves the oxidation of propionate and butyrate to acetate which can be utilized by the methanogens. The group of microorganisms responsible for this step are the acetogenic bacteria.

The thermodynamics of these acetogenic processes are unfavorable. In Table 1 the reactions that have been proposed (13) for the acetogens are listed. The ability for oxidation of propionate and butyrate has been rationalized by a syntrophic association between the acetogenic and methanogenic populations. Bryant (13), has explained this association by the maintance of a low H_2 concentration, thus permitting the oxidation of the substrates, e.g. propionate to produce acetate and H_2 (see Table 1). Studies by Bryant have demonstrated that syntrophic associations between ethanol catabolizing (10,12) and butyrate catabolizing (13) bacteria with methanogens exist. It has also been shown (46) that similar associations exist between lactate catabolizing bacteria and the methanogens. There has been no direct evidence (13) supporting any associations between the propionate catabolizing acetogens and methanogens. It has been shown by Smith (59) that