FACTORS AFFECTING GROWTH YIELDS OF MICRO-ORGANISMS IN THE RUMEN[1]

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The ruminal production of microbial cells is a function of energy availability to the microorganisms in the rumen and the efficiency of cellular synthesis. The efficiency of coil synthesis a (gm cell yield/mole of ATP or Y_{ATP}) tends to he constant in batch type cultures. In continuous cultures, the specific growth rate has a marked effect on Y . Other factors influencing Y in continuous cultures are discussed. The rumen can be likened to a continuous microbial culture;'however, the ruminal fermentation is not in steady state (as found in continuous culture system) with respect to bacterial outflow rat., bacterial cell members and substrate concentrations Problems in appraising the rumen fermentation solely on the basis of the continuous fermentation model are elucidated.

Key Words: Microbial growth yields, efficiency of cell synthesis, continuous culture

The ruminal fermentation is a coupled process between carbohydrate degradation, volatile fatty acid (VFA) production and concomitant ATP generation and the process of microbial cell synthesis from nitrogenous precursors (mainly NH_3-N) and other needed substrates, such as carbon skeletons, sulphur and others (Bergen and Yokohama 1977). The end-products of this ruminal fermentation which are used by the animal are the VFA and microbial cells (protein). The ruminant utilizes the VFA directly as an energy source, whereas the microbial cells are the animals primary source of protein (amino acids) and B complex vitamins (Hungate 1966).

It seems advantageous to have a close couple between ATP generation and cell growth in the rumen to use all the available energy in this thermodynamically limited anaerobic system (Bergen and Yokohama 1977). Factors impeding ruminal organic matter digestion or a lack of nitrogenous precursors will both lower VFA production and cell yield. Not all fermentations are well coupled; for instance the fermentation of lactose by *L. bulgaricus* results in lactic acid but little cell growth. This organism must dissipate the ATP generated in the lactose fermentation in some non-utilizable manner (Tempest 1978).

Energetics in the Rumen - An Overview

In the main, the combined activity of the ruminal microbiota ferments carbohydrates via glycolysis and then recharges reduced electron carriers by transmitting the reducing equivalents (or metabolic hydrogen) to specific carbon compounds or electron acceptors that when reduced are the typical end-products of the fermentation (Hungate 1966). These reduced end-products are primarily acetic,

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propionic and butyric acids and methane (CH_4) (Hungate 1966). Fermentations in ruminants fed high roughage rations produce more acetate, while high cereal grain rations result in more propionate (Hungate 1966). The associated ATP yields of the various fermentation pathways have been well described as follows (Isaacson et al 1975):

End-product formed	Mole of ATP/mole of end-product
Acetate	2
Propionate	3
Butyrate	3
Methane	1

Considerable discussion has occurred over the years on the desirability of CH_4 as an electron sink for the ruminant. One view is that CH_4 represents a loss of metabolic H_2 and in the absence of methanogenesis, this H_2 will be transmitted to propionate with no loss of metabolic H_2 to the animal (Demeyer and Van Nevel 1975). If one compares the $\triangle$ G in CH_4 generation (from HCOO and H), or the reduction of fumarate to succinate (and ultimately propionate), in each case the energy change allows for the synthesis of 1 ATP (Thauer et al 1977). Hence, with no apparent loss of ATP generation and the conservation of metabolic H_2, suppression of CH_4 production and enhancement of propionate should be encouraged.

Despite the above, chemical inhibition of methanogenesis has interfered with rumen function and feed utilization. An excellent example of this is the depression of the net energy (NE) for ground maize when fed with a CH_4 inhibitor (Cole and McCroskey 1975). The question arises as to why the NE of feeds is depressed by direct CH_4 inhibition despite the high recovery of metabolic H_2 and enhanced propionate formation. A theoretical answer to this problem is as follows. Methanogenesis generates extra ATP in the rumen in two ways. $_{First, CH4}$ is a direct electron sink with the concomitant generation of 1 ATP (mole/mole). Secondly, reduced electron carriers (NADH) can be regenerated directly be hydrogenase reactions; the resulting H_2 is then fixed by the methanogens. This allows the organisms to recharge their electron carriers without utilizing the limited carbon compound electron acceptors. This process then results in a higher ATP yield per unit substrate (Hungate 1966; Demeyer and Van Nevel 1975). When methanogenesis is inhibited the partial pressure of H_2 is increased, decreasing the hydrogenase activity and hence electron carriers must transfer their reducing equivalents to carbon electron acceptors thereby lowering the ATP yield per unit of substrate (Demeyer and Van Nevel 1975).

The usual substrates for methanogenesis in the rumen are H_2 and CO_2; however, under conditions of low turnover in continuous culture, and sludge and sediment fermentations, acetate can be converted to CH_4 (Zeikus 1977), Whereas CH production from H_2 and CO_2 is exergonic with a free energy change ($\triangle$G) of -32 to -34

Kcal/mole of reactants, the acetate to methane conversion is only slightly exergonic (G -8.6 Kcal/mole) and it would appear that this reaction could not support growth of the dissimilatory organism (Zeikus 1977). Recent work with the methanogen, Methanosarcina, indicated that acetate metabolism can result in ATP generation, but that 5.26 moles of acetate would be required for each mole of ATP generated. Since more than 1 mole acetate is required for the generation of 1 mole ATP, it is extremely unlikely that substrate-level phosphorylation occurs during acetate metabolism (Smith and Mah 1978). The mechanism of acetate conversion to CH_4 appears to be a direct reduction of the C-2 (i.e. the methyl group) to CH_4 by the proton as demonstrated by isotope studies (Pine and Vishniac 1957). Thus:

$$^{14}CH_3COO + {}^{3}H^{+} \text{ ----------> } {}^{14}CH^{3}_{4} + CO_2; \quad G \text{ -8.6 Kcal/mole}$$

This dissimilation of acetate without a large enough free energy change to allow ATP synthesis in certain anaerobic ecosystems represents a loss in carbon and hydrogen from the ecosystem. In the ruminant such a dissimilation of acetate would represent a lose of VFA as well as an energy lose for microbial cell synthesis.

Bacterial Growth Yield

Microbiologists have related ATP generation from substrate breakdown and cellular growth. These efforts resulted in a term for yield, Y, defined as:

$$Y_{substrate} = \frac{\text{weight of bacteria formed}}{\text{weight of substrate used}}$$

In energy limiting batch cultures these Y_s values are generally found to be constant. Bauchop and Elsden (1960) derived the term YATP defined as:

$$Y_{ATP} = \frac{\text{g cells formed}}{\text{mole ATP}}$$

Using an anaerobe with a known fermentation pathway and ATP generation potential, grown in energy limiting batch culture, these workers found that the Y_{ATP} = 10.5. Other workers found similar values and it was thought that the Y_{ATP} represented a biological constant (Payne 1970). This concept was erroneous as indicated below (Stouthamer and Bettenhaussen 1973).

To ruminant nutritionists, the ability to predict ruminal microbial cell yield is extremely important. Hence, accurate Y values or efficiency of cell yield values (cell yield/ energy used) must be known. It is of interest to observe that the concept of Y_{ATP} was applied in a totally different manner by microbiologists. They found in their work with different organisms that it was easy to measure cell yield but often difficult to assess the extent of ATP generation from substrate degradation. These workers then used Y_{ATP} to predict ATP yield/unit substrate degraded from growth yield and Y values.

Hungate (1966) first suggested that there was an "upper limit for the efficiency of microbial cell synthesis in the rumen. This was based on the fact that the rumen system is anaerobic with a limit in total ATP production, and that this places a limit upon synthesis of microbial cellular material. Using estimated ATP yields in the rumen fermentation and Y ATP of 10.5, Hungate (1966) suggested that this limit was 15 g microbial cell synthesis or 10 g microbial protein per 100 g of organic matter digested in the rumen (DOMr). This limit provides a less than desired level of microbial protein for the host for average growth and maintenance. In high producing ruminants (ea. during lactation), the ruminal fermentation cannot provide adequate protein. These animals depend on dietary by-pass protein. Hungate's proposal led to extensive studies of ruminal microbial growth yields by either using short term rumen incubation techniques (total synthesis) or abomasal or duodenal passage studies (net synthesis). Values of net synthesis, ranging from 20 to 26 g microbial protein/100 g DOMR, were reported by Bucholtz and Bergen 1973). Although most values reported were somewhat higher than Hungate's limit, there has been considerable disagreement over which values to use for prediction purposes. Computer simulation efforts by this writer have shown that the use of the highest values vastly overestimates microbial cell yields, when compared to animal performance results. A further problem is the evidence for variation of cell yield efficiencies when it had previously been thought that such variations did not occur in anaerobic systems. Finally, there has been the contention that the ruminal DOM determinations are often incorrect and cause this apparent variation in cell yield efficiencies (Czerkawski 1978).

All these problems have refocused efforts on more basic approaches to understand the factors that may affect microbial cell yield.

Factors Affecting the Y_{ATP}

Energy (ATP) requirements for cellular synthesis can be calculated if the following parameters are known (Stouthamer 1977). (I) A set of equations that accurately describe the energy expenditure of biosynthetic reactions; and (ii) analysis of the macromolecular composition of the cells to be formed. The basic biological processes that have ATP expenditures are protein synthesis lipid synthesis, RNA, DNA synthesis, polysaccharide synthesis and transport gradient processes. By summing the energy (ATP) expenditures for the various biosynthetic activities, the theoretical maximum cell yield/mole ATP (Y MAX ATP) can then be calculated. The complexity of the media and the carbon and nitrogen sources can substantially lower predicted YATPMAX (Stouthamer 1977) (Table 1). This is especially true where ATP is use extensively for monomer synthesis. The various factors that strongly influence YATp of bacteria are listed in Table 2. In a continuous culture at steady state, the specific growth rate (~) of the organism is equal to dilution rate of culture medium (D) and this factor has a profound effect on Y ATP As organisms grow faster, maintenance energy needs per cell are much smaller and larger and larger fractions of the available ATP from the CHO dissimilation are used for cellular growth. Hence, Isaacson et al (1975) showed that for a mixed ruminal bacterial culture grown in continuous culture at low D (2%/hr), the fraction of ATP spent for maintenance was about 60%; whereas at D of 12%/hr the fraction of ATP spent for maintenance was about 15-20%. Energy uncoupling and futile cycles (Table 2) may be considered part of the maintenance energy and other factors that may markedly depress YATP values in vivo resulting in lower growth yields than expected. Observed Y ATP values are thus always lower

than YMAXATP. The difficulty lies in assessing all the factors that may modify YATp, to achieve a realistic value to predict cell yields in the rumen, and animal growth rates. To date the only feasible approach has been to measure YATp (or cell yield efficiency) directly for many different regimens.

Table 1 :
Medium carbon and N source and the Y_{ATP}^{MAX}[1]

Medium	Main carbon source	Organisms	Y_{ATP}^{MAX}
Complex (AA, Nuc Acids)	Glucose	E. Coli	31.9
Inorganic salts[2]	Glucose	E. Coli	28.8
Inorganic salts[2]	Lactate	E Coli	13.4
Inorganic salts[2]	Malate	E. Coli	15.4
Inorganic salts[2]	Acetate	E. Coli	10.0
Inorganic salts[2]	CO_2	E. Coli	6.5

[1] From Stouthamer (1977)
[2] NH+4 no ATP needed (transport free)

Table 2: Factors affecting Y_{ATP}[1,2]

Maintenance needs	+++
Specific growth rate	+++
Medium complexity	+++
Nature of "C" source	++
Nature of "N" source	++
Cellular composition	
(except storage cmpds)	+
Presence of energy requiring process other than formation of new cell material	++
Futile cycles	++
Storage cmpds: high yield/mole ATP	+++
Energy coupling	++

[1] From Stouthamer (1977)
[2] No of + signs indicate importance of factor

The rumen fermentation has been likened to a continuous culture in that there is a more or less continuous substrate supply, end-product removal and a buffering system to keep the fermentation active. A continuous culture system, referred to as the "chemostat" has been employed to study basic aspects of the rumen fermentation. This culture system controls cellular growth by the rate of substrate addition and fractional outflow (dilution rate) in a fixed culture volume. This system, once equilibrated. exhibits a steady state between the growth limiting substrate supply and microbial growth rate. Cellular concentrations over various growth rates stay constant and the concentration of the growth limiting substrate in the medium stays low, near the Ks for the substrate. In steady state, to maintain a constant bacterial concentration in the medium, the specific growth rate equals the dilution rate. As the dilution rate increases, cell yield (eg bacterial concentration x D) increases.

The chemostat theory also assumes that the rate of cellular anabolism regulates the rate of catabolism of energy substrates. This has been shown to be untrue. In P or S limiting continuous cultures of K aerogens the rate of substrate degradation was independent of specific growth rate (Tempest 1978). Thus, at low specific rates of growth, the catabolism of substrate was as high as at higher growth rates, and, the organism's regulation of metabolism was not related to biomass synthesis. The organism must, therefore, possess alternative means of dissipating (uncoupling) the derived energy (ATP) from substrate catabolism (Tempest 1978).

Table 3 presents some comparisons between a chemostat and the rumen fermentation. The main points to emphasize are that in the chemostat, cell and substrate concentrations are low (10 to 103 cells/ml, and substrate near the Ks), while in the rumen, cell numbers are at 108 to 1011 cells/ml, and energy and nitrogenous substrates are usually in excess of the Ks. Also, in a chemostat, there is perfect mixing in a single liquid phase, while in the rumen, there are also, at least, a

Table 3:
Comparison of a chemostat and the rumen fermentation

Factor	Chemostat	Rumen
Steady state	Yes	No
Bacterial concentration (x)	Constant	Fluctuate
Substrate concentration (s)	Constant	Variable over feeding cycle
Dilution rate (D)	Constant	Variable over feeding cycle
Ceil yield (Dx)	Constant	Not constant
Molar growth yield (y) at given D	Constant	Complex
Growth limiting nutrient	Any needed nutrient	Energy? NH_3
Continuous culture	Yes	Yes and No
Mixing of x and s	Perfect liquid	Particle; liquid phase

liquid-small particle phase and the particle phase. There are micro-organisms associated with each phase, although most bacteria are associated with the particle phase, The relative rumen outflow rate of each phase will effect the specific growth rate of these organisms, the maintenance needs, and hence the efficiency of cell yield (eg Y ATP)

Rumen fermentation has characteristics of both continuous and batch culture systems. It would appear that at low ruminal digesta flow rates, the rumen fermentation in many ways resembles a batch type culture and the efficiency of cell yield (YATP) is relatively low, and constant; whereas under dietary conditions that result in higher rates of digesta flow, the rumen fermentation would resemble a continuous culture with higher energetic efficiencies for biomass synthesis. The physiological range of digesta (DM) flow from the rumen is from 2%/hr to about 8%/hr.

Microbial cell synthesis is dependent on total ATP availability as well as the efficiency of ATP use for biomass production. Preliminary evaluation of this concept in the rumen showed that feeds associated with lower outflows rates (3X/hr; eg processed-grain rations) have a higher total ATP production but a lower Y ATP, while feeds that encourage higher outflow (6.4%/hr; eg organic acid treated, high-moisture maize) have a lowered ruminal DOM and less ATP production, but a higher YATP. When both factors are taken together, the actual yield of microbial biomass from the rumen may be similar under these divergent dietary regimes.

More research is needed to solve the problem of how to predict accurately and systematically, cellular biomass production in the rumen. This information is needed to made meaningful decisions regarding microbial non-protein nitrogen utilization, as well as for decisions regarding the manipulation or processing of dietary proteins (eg by-pass) when the extent of microbial cell yield cannot meet the host's amino acid requirements.

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