The yields of *Streptococcus faecalis* grown in continuous culture

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SUMMARY: *Streptococcus faecalis* has been grown anaerobically in continuous culture on a defined medium. Under these conditions it is possible to maintain (a) the glucose concentration, (b) the tryptophan concentration, and (c) the generation time at predetermined values, and to study the effects of changes in these variables on the culture. When growth was limited by the glucose supply, the glucose yield constant (dry weight of cells formed/weight of glucose used) was greater than when tryptophan was limiting. The glucose yield constant under conditions of tryptophan limitation progressively fell with increasing supplies of glucose until it reached a minimum value. Under conditions of tryptophan limitation and large excess of glucose, the amount of glucose used per unit weight of cells per unit time remained roughly constant irrespective of growth rate. It is concluded that the needs of cell synthesis in *S. faecalis* do not control the rate of glucose breakdown, i.e. the rate of the energy-yielding metabolism. At slow growth rates the end products of glucose metabolism included volatile fatty acids, which were not present, or present in small amounts, at faster growth rates.

In the course of his studies of the factors which control the growth of bacteria Monod (1942) established that, when the energy source is the limiting nutrient, the quotient—weight of cells produced/weight of energy source used—is constant irrespective of the concentration of the carbon source. This quotient is referred to as the growth yield constant (GYC). Monod also showed that there is a simple relationship between the specific growth rate and the concentration of the growth-limiting nutrient. The saturation constant, i.e. the concentration of the limiting nutrient at which the specific growth rate is half maximal, is very low and Monod obtained values of 4, 2 and 25 mg/l. for glucose, mannitol and lactose respectively, as carbon sources for *Escherichia coli*.

In all, Monod studied three organisms, *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhimurium*. Monod's findings have been repeatedly confirmed by other microbiologists using other organisms grown batchwise upon a wide range of energy sources and the constancy of the GYC of a given organism grown on a particular energy source can be taken as established. However, it is important to realize that the values obtained by the various workers apply solely to the particular conditions employed, i.e. to growth in batch culture with the substrate in question the factor limiting growth and all other essential nutrients present in excess. It is clear from what has been said above that, under these conditions, the specific growth rate is maximal for almost the whole period of growth.

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Monod's work made possible the development of both the theory and the practice of continuous culture techniques, cf. Monod (1950), Novick & Szilard (1950), Herbert, Ellsworth & Telling (1956). With this technique it is possible to grow an organism in a constant environment, at a predetermined rate and at a constant cell density. Further, any essential nutrient can be made the limiting factor at will. We have used this technique to study the GYC, first, under conditions where the energy source is the limiting factor with all other nutrients present in excess; secondly, under conditions where some other nutrient is limiting and the energy source is present in excess. Our object was to examine the relationship between the rate of growth and the rate of catabolism. If the rate of growth controls the rate of catabolism and if this control is absolute then the GYC will be constant whatever the concentration of the energy source, no matter whether it is present in excess or whether it is the limiting nutrient. If synthesis does not control catabolism it is to be expected that the organism will catabolize at its maximum rate, and, once the energy source has ceased to be the factor limiting growth, the GYC will decrease with increasing concentration of the energy source. The use of the continuous culture technique is essential for work such as this, for it is the only method at present available for growing cultures at a constant, predetermined rate, at a constant cell density and in a constant environment.

In order to facilitate the interpretation of the results both the organism and the condition employed had to be such that the energy source functioned mainly, if not exclusively, in this capacity with negligible amounts assimilated, and secondly, it was desirable that the catabolic pathway and hence the energy yield should be independent of the conditions. *Streptococcus faecalis* was chosen as our experimental organism. It is nutritionally very exacting and growing cultures ferment glucose by the Embden-Meyerhof pathway to lactic acid with a 90-95% yield (Gunsalus & Niven, 1942).

METHODS

Organism and medium

Streptococcus faecalis, NCTC 6783, was used in all the experiments. Stock cultures were kept on slants of glucose + yeast autolysate + chalk agar and transferred at 2-monthly intervals.

The medium employed for growing the organism in continuous culture contained: casein acid hydrolysate (Oxoid) 1 g.; $(\text{NH}_4)_2\text{SO}_4$ 0.2 g.; L-asparagine 0.1 g.; trisodium citrate 0.05 g.; sodium acetate (trihydrate) 0.2 g.; K_2HPO_4 0.75 g.; KH_2PO_4 0.75 g.; nicotinamide 100 μg .; pyridoxin 200 μg .; Ca pantothenate 100 μg .; riboflavin 100 μg .; thiamine 100 μg .; biotin 10 μg .; folic acid 10 μg .; adenine 2 mg.; uracil 2 mg.; stock salts solution, 1 ml.; dist. H_2O to 100 ml. The stock salts solution contained: conc. HCl 50 ml.; MgO 10 g.; CaCO_3 2 g.; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 5.6 g.; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 1.44 g.; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 1.11 g.; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.25 g.; $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ 0.28 g.; H_3BO_3 0.062 g.; dist. H_2O to 1000 ml. This solution was prepared by adding the HCl to approx. 200 ml. dist. H_2O dissolving the MgO and CaCO_3 in that order, heating if necessary,

followed by the remaining salts and dist. H₂O to volume. DL-Tryptophan was added to the medium in concentrations varying from 0.8 to 15 µg./ml., the concentration depending on whether this compound was used as a growth-limiting factor or not. Glucose was sterilized by filtration in a 50%, w/v, aqueous solution and added aseptically to the sterile medium. Acetate was omitted from the above medium in those experiments in which it was necessary to estimate volatile fatty acids (VFA) formed during growth. The medium was sterilized in 9 l. quantities by autoclaving for 90 min. at 15 lb. pressure.

Continuous culture apparatus

General description. Two assemblies were used in the course of this work. One of these was designed and constructed by Dr D. Herbert of the Microbiological Research Establishment, Porton, England, the other was designed by one of us (R.F.R.). The Sheffield model differs from Herbert's mainly in the design of the growth vessel. The two models have the following features in common. The nutrient medium is supplied to the culture by means of a Sigma-motor peristaltic pump and Revco Zeromax speedchanger (Sigmamotor Inc., Middleport, N.Y., U.S.A.), the rate of medium flow being measured by the rate of filling of a graduated tube as described by Elsworth, Meakin, Pirt & Capell (1956). The medium reservoir, a 10 l. glass bottle, was replenished when necessary by connecting it to a second container filled with sterile medium and transferring the fresh medium by means of sterile compressed air. The volume of medium in the culture vessel was kept constant by means of an overflow, which served also as the escape port for any gas supplied to the culture. The effluent from the growth vessel was directed either to a storage reservoir or to a small container for the purpose of sampling, and the storage reservoir could be emptied while the apparatus was in operation by connecting it to an empty sterile container. Silicone rubber tubing was used throughout. The apparatus was sterilized by autoclaving and for this purpose it was broken down into three sections (medium reservoir, growth vessel, and storage reservoir) which were later rejoined with aseptic precautions.

Growth vessels. The apparatus designed by Herbert contained a growth vessel of approximately 90 ml. working capacity which was fitted with a water jacket to control temperature. Mixing was achieved by a free-spinning magnetic stirrer.

The detailed layout of the growth vessel used in the Sheffield apparatus is shown in Figs. 1 and 2. The flanged glass container made for us by Moncrieff, Alloa, Scotland, is provided with two outlets: (*A*) serves as an overflow and is so placed that the working volume is kept at approximately 300 ml.; the second (*B*) is an additional sampling point. The stirring magnet (*C*) is mounted in a nylon holder (*D*) which is kept in position by a shaft projecting from the lid. The nylon holder is so shaped that a point contact is made between it and the bottom of the vessel. The stainless steel baffles (*E*) are mounted on a single flat strip of stainless steel. When the flat strip is bent into the circular shape of the growth vessel, its natural spring is sufficient to hold the baffles in place. The metal lid (*F*) and all the attachments save the heater shield, *vide infra*,

were made of brass and heavily plated with chromium. In between the metal lid and the glass flange is placed a neoprene gasket (*G*). The lid is held in place by means of a clamp, not shown in the figures and the joint so obtained is gas-tight. Three tubes, welded into the lid and sealed at the end projecting into the vessel carry (*H*), a thermostat (Sunvic Stem Thermostat, TS. 3, Sunvic Controls Ltd., London); (*I*), a 50 W. heater and (*K*) a thermometer. After sterilization these tubes are filled with medicinal paraffin and the thermostat, heater and thermometer immersed therein. The pocket containing the heater is surrounded by a stainless steel shield (*J*) which prevents splashes from the culture reaching those parts above the surface of the liquid. Without the shield occasional drops of culture bounced on to the upper part of the heater pocket, (*I*), dried off and charred, so much so, that, at the end of a long run, the heater pocket was heavily encrusted.

Fig. 1

Fig. 2

Fig. 1. Diagram (side view) of the growth vessel used in the Sheffield apparatus: *A*, overflow; *B*, additional sampling point; *C*, magnetic stirrer mounted in nylon holder *D*; *E*, stainless steel baffles; *F*, lid; *G*, neoprene gasket; *H*, thermostat holder; *I*, holder for 50 W. heater with shield *J*; *K*, thermometer holder; *L*, medium inlet tube; *M*, air inlet tube.

Fig. 2. Diagram (top view) of the growth vessel used in the Sheffield apparatus and lettered to correspond with Fig. 1 and with the description in the text.

The lid is further provided with five inlets, one of which (*L*) served as medium inlet, and a second (*M*) as air inlet. During the course of these experiments no use was made of the other three openings which were sealed off.

Gas supply. To ensure anaerobic conditions during growth, nitrogen from a cylinder was passed through the culture at a rate of approximately 15–20 ml./min.

Growth in continuous culture

The vessel was filled with sterile medium and the heater turned on. The thermostat was set at 37° for all experiments and, when this temperature was reached, the medium was inoculated and the culture grown batchwise until the culture density reached its maximum. In the course of an experiment the temperature varied between 36.5° and 37.5°. Once a steady state was established the cell density remained practically constant so long as the flow rate and the composition of the medium remained unchanged (Fig. 3). The duration of any given steady state varied from experiment to experiment, but was never shorter than 15 hr., and in some cases was as long as 90 hr. Continuous operation of the apparatus, examining one steady state after another, has been carried on for up to 3 weeks, though usually the apparatus was dismantled, cleaned and re-sterilized at more frequent intervals. The dilution rate, D —defined as the flow-rate in ml. hr.⁻¹/vol. of culture in the growth vessel—is taken as an index of the growth rate. Under steady-state conditions D is related to μ (the specific growth rate) and t_d (the doubling time of the culture) by the equations

$$D = \mu = (\log_e 2)/t_d$$

Fig. 3. Steady-state growth of *Streptococcus faecalis* with glucose as the growth-limiting factor. Dilution rates: A, 0.43; B, 0.39; C, 0.182 hr.⁻¹. A and B with Porton apparatus, C with Sheffield apparatus.

Estimations

Sampling. For the estimation of bacterial dry weight, samples were collected and examined immediately (*vide infra*). Samples for chemical estimations were collected in tubes containing 0.5 ml. 50 % (v/v) H₂SO₄.

Bacterial dry weight. Indirect determinations of dry weight were made by measuring the optical density of samples in a Hilger Spekker absorptiometer using a 1 cm. cell and a neutral grey filter and relating the readings to a standard curve. For direct determinations samples were centrifuged and washed twice with an accurately prepared 1 % (w/v) NaCl solution. The organisms thus washed were then resuspended in a measured volume of the NaCl solution dried for 24 hr. at 110°, and the residue weighed.

Lactic acid. The methods of Friedemann & Graessre (1933) and of Elsdén & Gibson (1954) were employed.

Glucose. Glucose in media, cultures, and cells was determined by the anthrone method of Trevelyan & Harrison (1952).

Volatile fatty acids. Total volatile fatty acids were separated by steam distillation in a Markham still and the distillate titrated with NaOH in a stream of CO₂-free air. Chromatograms were prepared as described by Hiscox & Berridge (1950).

Ethanol. Ethanol was detected by measuring the reduction of diphosphopyridine nucleotide (DPN) in the presence of yeast alcohol dehydrogenase and NaHSO₃. The enzyme preparation, kindly supplied by Dr S. Sentheshan-muganathan, had negligible lactic dehydrogenase activity.

RESULTS

pH values of cultures. The growth medium was strongly buffered and after autoclaving had a pH value of 6.5. The pH values during steady-state conditions varied with the glucose concentration in the medium but never fell outside the range pH 6.4–5.7.

Relationship between bacterial dry weight and optical density of the culture

Herbert (1959), Pirt (1957) and Perret (1958) have described changes in the size of cells of *Escherichia coli*, *Aerobacter cloacae* and *Bacillus megaterium* strains when grown at different rates in continuous culture. Since the concentration of cells was measured by a photometric method and since light scattering is a function of particle size it was necessary to study the effect of growth rate on the ratio, optical density/bacterial dry weight, the dry weight being measured by direct weighing. The results shown in Fig. 4 indicate that with *Streptococcus faecalis* the optical density/dry weight ratio does not vary significantly with the growth rate, at least over the range of growth rates examined.

Fig. 4. Relation between the ratio optical density/bacterial dry weight and dilution rate during the steady-state growth of *Streptococcus faecalis*. Optical density measured in a 1 cm. cell.

Growth at constant flow rate and tryptophan concentration and varying glucose concentration

The strain of *Streptococcus faecalis* used required tryptophan for growth and both this amino acid and glucose were supplied to the culture in low

concentrations, the other nutrients being in large excess. At the start of each experiment, the concentrations of both glucose and tryptophan were such that no matter which of the two was actually growth limiting, small changes in the concentration of the other reversed the position. The flow rate and tryptophan concentration were subsequently held constant while the glucose concentration in medium was varied. A steady state was achieved for each glucose concentration before examining the next.

When glucose is growth limiting its concentration in the culture under steady-state conditions will be very low, independent of its concentration in the inflowing medium, and constant for so long as the growth rate remains constant (Monod, 1950; Novick & Szilard, 1950; Herbert *et al.* 1956). Raising the glucose content of the incoming medium will increase the cell density in the culture only so long as the sugar is growth limiting. When the culture reaches a density such that the tryptophan becomes the limiting factor, raising the glucose in the incoming medium will no longer lead to increases in bacterial numbers. If the amount of cell material formed per unit weight of glucose metabolized (glucose yield constant GYC) remains the same whether glucose or tryptophan are rate-limiting for growth, glucose will accumulate in the culture as soon as its concentration is raised to the level where tryptophan becomes the limiting factor. On the other hand the concentration of lactic acid, the end product of glucose catabolism, in the effluent will remain unchanged once the stage of tryptophan limitation is reached.

Results for dilution rates (D) = 0.312, 0.22 and 0.43 hr⁻¹ are shown in Figs. 5–7 respectively. When glucose was the growth-limiting factor the culture density increased with rising glucose concentration; when tryptophan was limiting the culture density remained constant with rising glucose. That tryptophan was indeed the limiting factor is shown more directly by the rise in the density of the culture following the addition of more tryptophan to the medium (Fig. 6).

At these dilution rates the GYC varied with changing glucose concentration. That glucose added after tryptophan became limiting was utilized is shown both by the rise in the lactic acid concentration and by the low sugar concentration in the effluent. With increasing supply of glucose, the GYC fell until a point was reached where the rate of glucose inflow exceeded the rate of fermentation and the sugar accumulated in the culture.

Growth at constant glucose and tryptophan concentration and varying growth rate

In subsequent experiments, the amounts of tryptophan and glucose in the medium were so adjusted that the tryptophan was growth limiting and glucose present in amounts sufficient to saturate the fermentation system of the organism. The accumulation of the sugar in the culture under steady-state conditions served as the criterion of whether glucose was indeed present in such excess. Steady states were then achieved at various dilution rates.

The rate at which cells growing in these steady states metabolized glucose was calculated from the concentration of glucose in both medium and culture,

the rate medium was supplied to the culture and the culture density. Since glucose is present in the culture the rate so calculated represents the maximum rate of fermentation of glucose by the cells under these particular conditions. Figure 8 shows that it is roughly constant and independent of the growth rate.

Fig. 5. Steady-state growth of *Streptococcus faecalis* at constant growth rate and tryptophan concentration and different glucose concentrations. Dilution rate = 0.312. The culture density and the glucose and lactic acid concentrations were determined in five steady states. The glucose concentration in the medium for each of these steady states is indicated below the histogram.

Fig. 6. Steady-state growth of *Streptococcus faecalis* at constant growth rate and tryptophan concentration and different glucose concentrations. Dilution rate = 0.22. The culture density and the glucose and lactic acid concentrations were determined in seven steady states. The glucose concentration in the medium for each of these steady states is indicated below the histogram. During the first three steady states (left of arrow) the medium contained 0.8 µg./ml. DL-tryptophan, for the last four steady states this was raised to 1.3 µg./ml.

Thus catabolism of glucose proceeds at a rate unrelated to the rate of synthesis when growth is controlled by tryptophan.

The GYC's found under these conditions are shown in Fig. 9. A linear relationship is seen to exist between yield and growth rate, more efficient utilization of the sugar occurring the faster the growth rate. Since at least one bacterial species, *Escherichia coli*, has been shown to store greatly increased

Fig. 7. Steady-state growth of *Streptococcus faecalis* at constant growth rate and tryptophan concentration and different glucose concentrations. Dilution rate = 0.43. The culture density and the glucose and lactic acid concentrations were determined in five steady states. The glucose concentration in the medium for each of these steady states is indicated below the histogram.

Fig. 8

Fig. 9

Fig. 8. Rate of glucose metabolism of *Streptococcus faecalis* cells growing at various dilution rates with tryptophan the limiting factor and glucose present in excess. The rate of glucose metabolism was calculated from the rate of medium supply, the difference in the glucose concentration between the medium and culture and the culture density.

Fig. 9. Relationship between the glucose yield constant (dry weight cell material formed per unit weight of glucose metabolized) and dilution rate of *Streptococcus faecalis* in steady states with tryptophan as the factor limiting growth and glucose present in excess.

amounts of polysaccharides in the cells when grown at slow rates in continuous culture with the nitrogen source as the limiting factor (Holme 1957), the possibility of such storage in *Streptococcus faecalis* was investigated. Estimation of total carbohydrate in cells from cultures of differing GYC's showed only slight variation (Table 1) and the differences observed are certainly not sufficient to affect the GYC.

Table 1. Comparison of the carbohydrate content of cells growing under steady-state conditions and the glucose yield constants (cell dry weight formed/unit weight glucose used) found for these steady states

Dilution rate	Glucose yield constant	Total cell carbohydrate as % of cell dry weight
0.265	0.241	11.8
0.265	0.14	10.8
0.265	0.12	15.6
0.265	0.12	11.9
0.305	0.16	8.5
0.49	0.218	7.9
0.61	0.215	7.2
0.716	0.257	7.3

Catabolic end products at various growth rates

Although it was clear from experiments such as described in Figs. 5-7 that lactate was a major end product of glucose metabolism and continued to accumulate after tryptophan became limiting, the amounts of lactate formed per unit of glucose metabolized were lower at slower growth rates than at faster ones and there was less than the 90-95 % conversion described by Gunsalus & Niven (1942). In Fig. 10 the percentage of the glucose disappearing which was recovered as lactic acid is plotted against growth rate for those steady states in which tryptophan was limiting and glucose in large excess. The values obtained vary with the growth rate. Gunsalus & Niven (1942) observed low yields of lactic acid in culture of *Streptococcus faecalis* growing at pH levels higher than 6.5, part of the glucose being converted to volatile fatty acids and ethanol. Since in continuous culture experiments low yields of lactate were obtained at pH values less than those where recovery was over 90 % (Fig. 10) we think that the variation in the yield of lactate in these experiments is a function of the growth rate rather than the hydrogen-ion concentration of the medium.

In order to facilitate the estimation of VFA acetate was omitted from the medium. Steady states at various growth rates were obtained with glucose as the growth-limiting factor. In the absence of acetate, the maximum rate of growth of the organism is much slower than in its presence, and dilution rates greater than $D = 0.4$ could not be examined. However, over the range investigated, the results (Table 2) resembled those found in the medium containing acetate and with tryptophan limiting growth. Less lactic acid was produced from glucose at slow than fast growth rates. Volatile fatty acids

were formed and the amounts found show an inverse relationship with the growth rate. Ethanol was not detected. The pH values under steady-state conditions again indicate (Table 2) that variation in the end products depended on growth rate rather than on pH. Paper chromatography showed the absence of any VFA higher in the series than formic and acetic. The formation of formic acetic acids is, however, a reaction known to occur in streptococci (White, Steel & Pearce, 1955; Gunsalus & Niven, 1942).

Fig. 10. Relation between percentage of glucose metabolized which was recovered as lactic acid (●), pH level of cultures (○) and dilution rate during steady-state growth of *Streptococcus faecalis* with tryptophan limiting growth and glucose present in large excess.

Table 2. *End products of the glucose metabolism of Streptococcus faecalis at various growth rates. Acetate omitted from the medium; growth-limiting factor glucose*

Dilution rate	pH	Glucose used (μ moles/ml. culture)	Lactic acid formed (μ equivalents/ ml. culture)	Volatile fatty acid formed (μ equivalents/ ml. culture)
0.086	6.31	17.6	19.5	24.1
0.154	6.15	17.6	22.0	18.3
0.246	5.91	17.6	24.8	14.8
0.302	5.7	17.1	28.6	10.2
0.392	6.3	7.6	13.1	4.2

DISCUSSION

Streptococcus faecalis has complex nutritional requirements, and uses glucose almost exclusively as an energy source. Thus Gunsalus & Niven (1942) reported a 90–95% conversion of glucose to lactic acid during the growth of this organism and Bauchop (private communication) found that when *S. faecalis* was grown upon G-C¹⁴-glucose only a small amount of the glucose carbon appeared in the cells. When this organism is grown in batch culture with glucose as the factor limiting growth the amount of growth is

proportional to the amount of glucose in the medium and the GYC with respect to glucose is constant (cf. Sokatch & Gunsalus, 1957; Bauchop, 1958).

Streptococcus faecalis has now been grown in continuous culture over a range of doubling times (cf. Herbert *et al.* 1956). The medium used contained a fixed amount of tryptophan and the glucose content was varied; thus, for each doubling time studied, growth was first limited by glucose and then by tryptophan and for each steady state the cell density, the glucose content and the lactate content of the outflowing medium were measured. The results show that, when the organism is grown with tryptophan as the limiting factor, increasing the glucose concentration of the medium results in an increase in the amount of glucose catabolized and hence of lactic acid formed. This means that under these conditions, i.e. tryptophan limiting and excess glucose, the GYC with respect to glucose varies inversely with the glucose concentration. In other words, the rate of catabolism is not then controlled by the rate of growth.

It would appear, however, that the growth rate has a qualitative effect on catabolism for, when the doubling time was long, the amount of lactic acid formed per mole of glucose used decreased, and lower volatile acids, acetate and probably formate, were produced instead. Gunsalus & Niven (1942) observed that similar organisms produced these two acids and ethanol from glucose when the pH of the medium was above 6.5. Whether the appearance of these compounds was the result of a direct effect of the pH on the catabolic enzyme systems, or whether it was due to a slower growth rate in the more alkaline medium is not clear. It would seem that this phenomenon would repay further investigation.

The fact that the rate of catabolism is not controlled by the rate of growth has implications which warrant further discussion. In the conversion of glucose to lactic acid via the Emden-Meyerhof pathway both ATP and ADP play essential parts; at the same time the process brings about a net synthesis of two moles ATP/ mole of glucose fermented. It is clear that, unless there is a mechanism for the continuous conversion of the ATP so synthesized back to ADP, the whole process of fermentation will stop due to a lack of ADP. The full importance of a continuous supply of ADP for maintenance fermentation was made apparent by Meyerhof (1945) in his study of the factors responsible for the Harden-Young reaction in yeast preparations. In the growing cell, the ADP supply is normally maintained by the utilization of ATP for synthetic and other forms of work. Since *Streptococcus faecalis*, grown under conditions of tryptophan limitation, exerts no control over the rate of fermentation of glucose, it is clear that there must be some other mechanism for the disposal of any surplus ATP which may be formed. The magnitude of this may be gauged from the results given in Figs. 5-7. It will be seen from Fig. 5 that, under conditions of glucose excess, approximately 4 times the amount of glucose was fermented per unit weight of cells produced as under conditions of glucose limitation. The obvious way of disposing of ATP would be through the action of an adenosine triphosphatase.

White & Munns (1951) studied the growth of *Saccharomyces cerevisiae* and

observed that when the energy source, sucrose, was the limiting factor for growth, the GYC with respect to sucrose was greater than when other factors were limiting. Karush, Iacocca & Harris (1956) have studied the growth of *Streptococcus pyogenes* in continuous culture under conditions where some nutrient other than the energy source limited growth. Their experiments were mainly concerned with the limitation of growth rate by the hydrogen-ion concentration. Only culture density and total acidity were measured. With pH as the growth-limiting factor, the rate of catabolism decreased as the growth rate was decreased. However, the rate of catabolism decreased more slowly than the growth rate and the yield constants were lower at the slow growth rates. These observations resemble those described above for *Streptococcus faecalis*.

The results of the work described above have some practical implications. First, it would seem that when the objective is to produce maximum cell yields/mole of energy source used, the energy source should be made the factor limiting growth. Secondly, when a catabolite is the end-product required, the energy source should be present in excess and some other essential nutrient made the limiting factor. The choice of which nutrient might well prove important since it is well known that specific nutritional deficiencies can lead to the accumulation of particular catabolites, e.g. the accumulation of pyruvate under conditions of thiamine deficiency. Thirdly, it is clear that precise control of the concentration of the factor limiting the growth of a culture used for production in the way outlined above can only be achieved by the method of continuous culture.

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