J. gen. Microbiol. (1960), **23**, 457–469
Printed in Great Britain

The Growth of Micro-organisms in Relation to their Energy Supply

By T. BAUCHOP* and S. R. ELSDEN

Agricultural Research Council Unit for Microbiology, Department of Microbiology, The University of Sheffield, Sheffield 10

(*Received* 4 *May* 1960)

SUMMARY

When *Streptococcus faecalis* was grown anaerobically in a complex medium containing D-glucose, D-ribose or L-arginine as energy source the dry wt. of organism produced was proportional to the concentration of the energy source in the medium. However, *S. faecalis* will not grow in a defined medium with arginine as the energy source unless glucose is present at the same time. The anaerobic growth of both *Saccharomyces cerevisiae* and *Pseudomonas lindneri* was proportional to the concentration of glucose in the medium and the yield coefficient—defined as g. dry wt. organism/mole glucose—of the former was the same as that of *S. faecalis* grown upon glucose and approximately twice that of *P. lindneri*. Calculation of the g. dry wt. organism/mole adenosine triphosphate synthesized for these three organisms gave values ranging from 12·6 to 8·3 with an average of 10·5. These results suggest that, under anaerobic conditions, the yield of *S. faecalis*, *S. cerevisiae* and *P. lindneri* was proportional to the amount of ATP synthesized. When *Propionibacterium pentosaceum* was grown anaerobically with glucose, glycerol or DL-lactate as energy source there was, in all three cases, a linear relationship between the dry wt. of organisms produced and the concentration of the energy source in the medium. The values of the yield coefficients obtained were compatible with the formation of approximately 4 mole ATP/mole glucose, 2 mole ATP/mole glycerol and 1 mole ATP/mole lactate.

INTRODUCTION

Over the past twenty years an enormous literature has grown up around the quantitative aspects of microbial nutrition. The majority of the papers published have been concerned with the relationship between the growth of an organism, usually estimated indirectly either by optical methods or by the titration of an acidic end-product, and the amount of some essential nutrient, other than the energy source, in the medium. The fact that there is a linear or almost linear relationship between growth and the amount of a particular nutrient has led to the development of highly sensitive specific methods for the quantitative estimation of amino acids, purines, pyrimidines and the B group of vitamins. In contrast there has been very little work on the relationship between growth and the amount of the energy source in the medium. The most complete study of recent years was that of Monod (1942) who studied the growth of three organisms—*Bacillus subtilis*, *Escherichia*

* Present address: The Wellcome Laboratories of Tropical Medicine, Euston Rd, London, N.W. 1.

coli and *Salmonella typhimurium*. These organisms were grown anaerobically on a mineral medium with a wide range of carbohydrates as energy source, and as long as the energy source was the factor limiting growth, the dry weight of organism produced was proportional to the weight of energy source added. It is of particular interest that for a given carbohydrate the three organisms produced approximately the same dry wt. yields.

DeMoss, Bard & Gunsalus (1951) studied the growth of *Streptococcus faecalis* and *Leuconostoc mesenteroides* and expressed their results as dry wt. organism/mole energy source provided. Both of these organisms have complex nutritional requirements and both obtain their energy for growth by the anaerobic catabolism of carbohydrates. Thus, *S. faecalis* converts glucose to lactic acid and *L. mesenteroides* to an equimolecular mixture of lactic acid, ethanol and carbon dioxide. The yields of the fermentation products were almost quantitative, suggesting that very little of the glucose was assimilated and that it was used almost exclusively as an energy source. This is in marked contrast to the aerobes studied by Monod (1942) which, since they were grown upon a simple mineral medium, used a considerable portion of the energy source for the synthesis of cell material. DeMoss *et al.* (1951) (see also Sokatch & Gunsalus, 1957) found that with both *S. faecalis* and *L. mesenteroides* there was a linear relationship between the dry weight of organism produced and the amount of glucose added, and that with glucose as the energy source the yield of *S. faecalis* was significantly greater than that of *L. mesenteroides*. They concluded from these results that, because both organisms used glucose mainly as an energy source, and because growth was proportional to the amount of energy supplied, *S. faecalis* obtained more energy/mole glucose than did *L. mesenteroides* and that the latter organism fermented glucose by a mechanism other than the Embden–Meyerhof pathway. This is a conclusion of considerable importance. The work of Gunsalus & Gibbs (1952) and more recently of Hurwitz (1958) has shown that, not only does *L. mesenteroides* ferment glucose by a new pathway involving xylulose-5-phosphate (Heath, Hurwitz, Horecker & Ginsberg, 1958) as the key intermediate, but also that the process yields only 1 mole of adenosine triphosphate (ATP)/mole of glucose, as compared with the 2 mole of ATP/mole of glucose produced by the Embden–Meyerhof pathway which is the fermentation mechanism of *S. faecalis*. From this it would appear that the growth of *S. faecalis* and *L. mesenteroides* is related to the amount of ATP produced.

In order to study further the relationship between the growth of *Streptococcus faecalis* and the energy supply we have measured the weight of organism produced when it was grown on D-glucose, D-ribose and L-arginine. Arginine was studied because it was observed that addition of this compound to the medium increased the yield of organism. The work of Akamatsu & Sekine (1951) established that *S. faecalis* converts arginine quantitatively to ornithine with citrulline as the intermediate and Knivett (1954*a*) subsequently found that the breakdown of citrulline was coupled with the synthesis of ATP. Thus, in terms of mole ATP formed/mole of substrate, arginine yields half as much ATP as glucose. In addition to these experiments the growth of *Saccharomyces cerevisiae* and *Pseudomonas lindneri* on glucose was measured; these two organisms were selected because both ferment glucose to ethanol and carbon dioxide. The former uses the Embden–Meyerhof pathway which gives 2 mole ATP/mole glucose and the latter the Entner–Doudoroff

pathway (Gibbs & DeMoss, 1954) which gives one mole ATP/mole glucose (see Elsden & Peel, 1958). Finally, the growth of *Propionibacterium pentosaceum* was measured on glucose, glycerol and DL-lactate. A preliminary account of this work has been published (Bauchop, 1958).

METHODS

Organisms. The following cultures were used: *Streptococcus faecalis* (NCTC 6783) a laboratory strain of *Saccharomyces cerevisiae*; *Propionibacterium pentosaceum* strain E.2.1. from the collection of Dr C. B. van Niel; *Pseudomonas lindneri* ATCC No. 10988, NCIB No. 8938.

Media

Stock salt solution (*S.S.S.*) MgO, 10·75 g.; $CaCO_3$, 2·0 g.; $FeSO_4.7H_2O$, 4·5 g.; $ZnSO_4.7H_2O$, 1·44 g.; $MnSO_4.4H_2O$, 1·12 g.; $CuSO_4.5H_2O$, 0·25 g.; $CoSO_4.7H_2O$, 0·28 g.; H_3BO_3, 0·06 g.; HCl (concn.), 51·3 ml. This mixture was made up in distilled water to 1 l.

Streptococcus faecalis *partially defined medium.* 10 g. casein hydrolysate (Oxoid) were dissolved in 100 ml. distilled water, 15 ml. N-NaOH added and the mixture allowed to stand for 15 min. The precipitate which formed was filtered off and 15 ml. N-H_2SO_4 added to the filtrate. The following mixture was then added to this solution: KH_2PO_4, 7·5 g.; K_2HPO_4, 7·5 g.; $(NH_4)_2SO_4$, 2 g.; adenine, 20 mg.; DL-tryptophan, 10 mg.; S.S.S., 5 ml.; asparagine, 1 g.; L-cysteine, 0·1 g.; nicotinamide, 1 mg.; Ca pantothenate, 1 mg.; riboflavin, 1 mg.; pyridoxin, 2 mg.; folic acid, 0·1 mg.; biotin, 0·1 mg.; adjusted to pH 6·6–6·8 and the volume made up to 1 l. with distilled water.

Saccharomyces cerevisiae *medium.* This medium was a modification of that used by White & Munns (1951): casein hydrolysate (Oxoid), 10 g.; Na_2HPO_4, 2 g.; S.S.S., 5 ml.; thiamine HCl, 0·2 mg.; riboflavin, 0·1 mg.; nicotinamide, 5·0 mg.; *p*-aminobenzoic acid, 0·3 mg.; pyridoxin, 1·0 mg.; Ca pantothenate, 0·5 mg.; *meso*-inositol, 50 mg.; biotin, 6 μg.; adjusted to pH 5·0 and made up to 1 l. with distilled water. For anaerobic growth this medium was supplemented with ergosterol (Andreasen & Stier, 1953): Ergosterol (63·8 mg.) and Tween 80 (6·5 ml.) were boiled with absolute ethanol until the ergosterol was dissolved, and the solution made up to 25 ml. with absolute ethanol; 3 ml. of this solution were added to 150 ml. of the medium at the time of the inoculation.

Propionibacterium pentosaceum *medium.* This was a modified version of the defined medium of Delwiche (1950). 5 g. Casein hydrolysate (Oxoid) were dissolved in 100 ml. distilled water, 15 ml. N-NaOH added and the mixture allowed to stand for 15 min. The precipitate which formed was filtered off and 15 ml. N-H_2SO_4 added to the filtrate. The following additions were then made: $CH_3COONa.3H_2O$, 8 g.; K_2HPO_4, 7·5 g.; KH_2PO_4, 7·5 g.; S.S.S., 5 ml.; L-cysteine, 50 mg.; sodium thioglycollate, 0·2 g.; DL-tryptophan, 0·1 g.; adenine, 10 mg.; guanine, 10 mg.; uracil, 10 mg.; xanthine, 10 mg.; Ca pantothenate, 1 mg.; thiamine HCl, 1 mg.; biotin, 0·5 mg.; *p*-aminobenzoic acid, 1 mg.; riboflavin, 4 mg.; adjusted to pH 6·6–6·8 and made up to 1 l. with distilled water.

Pseudomonas lindneri *medium.* This consisted of 1% (w/v) Bacto Peptone and 1% (w/v) Difco yeast extract.

Cultivation and harvesting of organisms

Direct dry weight measurements. Conical flasks (250 ml.) were prepared containing 150 ml. medium and a test-tube containing 3 ml. of 15% (w/v) pyrogallol was placed in each flask. The flasks were sterilized by autoclaving at 15 lb. sq.in. for 15 min. The required amounts of glucose and inoculum were then added, and in the case of the yeast, the appropriate amount of ergosterol solution. Finally, 3 ml. of 10% Na_2CO_3 were added to the test-tube containing pyrogallol. The flasks were closed with rubber stoppers, sealed with paraffin wax, and incubated at the appropriate temperature. *Streptococcus faecalis* was grown at 37°; *Saccharomyces cerevisiae*, *Propionibacterium pentosaceum* and *Pseudomonas lindneri* were grown at 30°. When growth had reached completion the organisms were harvested by centrifugation, washed three times with 100 ml. distilled water, washed into beakers and dried at 100° to constant weight.

Dry weight by turbidity measurements. Organisms were grown in 6 × $\frac{3}{4}$ in. test-tubes containing 9 ml. medium. Substrates, inocula and other additions, where applicable, were made to a final vol. of 10 ml. A wad of absorbent cotton wool was then pushed down over the sterile test-tube plug and on this were placed 5 drops each of 15% (w/v) pyrogallol and 10% (w/v) Na_2CO_3, in that order. The tube was sealed with a rubber stopper and incubated at the appropriate temperature. Growth was measured turbidimetrically in an EEL nephelometer (Evans Electroselenium Ltd., Harlow, Essex) until it reached completion, when the optical density was read in a Spekker Absorptiometer, with an H 508 filter. The Spekker readings were converted into dry weights by reference to a standard curve relating optical density to dry weight.

Continuous culture apparatus. The apparatus used was that described by Rosenberger & Elsden (1960). Anaerobic conditions were obtained by aerating the culture vessel with oxygen-free nitrogen.

Analytical methods

Radioactivity measurements. Radioactivity was measured with an end-window Geiger–Muller counter. A sample of the uniformly labelled D-(^{14}C)-glucose (obtained from the Radiochemical Centre, Amersham, Buckinghamshire) solution was diluted with an equal volume of absolute ethanol, and counted directly by spreading 25 μl. of this solution containing 125 μg. glucose on brass disks (Ormerod, 1956) and drying under an infra-red lamp. Results, corrected for background, are given as counts/min. at infinite thinness.

Glycerol was estimated by the method of Burton (1957) with glucose as a standard (O'Dea & Gibbons, 1953).

Lactate was estimated by the method of Elsden & Gibson (1954).

Arginine dihydrolase was assayed by the method of Knivett (1954*b*).

Definition of terms used. To facilitate comparison of the results obtained with the various substrates and organisms we express our results as: g. dry wt. organism produced/mole substrate added. We call this ratio the yield coefficient, Y, with the substrate given as a subscript; thus $Y_{glucose}$ refers to the yield coefficient of an organism growing upon glucose as the energy source. The yield coefficient has, in the past, been given a variety of names, for example, the yield constant, the growth

yield constant, the molar growth yield. Our reasons for introducing a further term for this ratio are as follows. Herbert (1958) has shown that the value of Y decreases with decreasing growth rate. Since the growth rate is a function of the substrate concentration (Monod, 1942) it is clear that, in batch cultures, once this critical substrate concentration is reached, the value of Y will begin to decrease. However, the concentration of the substrate at which the growth rate begins to be affected is very low as compared to the initial substrate concentration (Monod (1942)), and in consequence the value of Y as defined above is not significantly affected. On the other hand, it seems to us inappropriate to call a ratio a constant when it is manifestly not constant under all conditions.

The ATP yield of a metabolic process is defined as: mole ATP produced/mole substrate used. Where both the yield coefficient Y and the ATP yield of the metabolic process involved are known it is possible to calculate the dry wt. of organism produced/mole ATP formed; the ratio so obtained we call Y^{ATP}.

RESULTS

Yield coefficients

All experiments were carried out under strict anaerobiosis and the media were so constituted that the energy source was the factor which limited growth. To ensure that this was the case the yields were measured over a range of substrate concentrations. Figure 1 shows plots of the growth yields of *Streptococcus faecalis* on the complex medium against the concentration of the substrates D-glucose, D-ribose and L-arginine, respectively. It will be noted from Figs. 1 and 2 that although there was a linear relationship between the wt. of *S. faecalis* produced and the concentrations of the three substrates, the curves did not pass through the origins. Rather, at zero substrate concentration, they intersected the ordinate at a positive value for dry wt. This could mean either, that over a range of substrate concentrations less than those tested, the slopes of the curves and hence the yield coefficients were greater than at higher substrate concentrations. Or, that there was present in the media a small but significant amount of an unknown energy source upon which the organism grew. We think that the latter is the correct explanation for there was always some growth in both the media used in the absence of an added energy source. The magnitude of this 'blank' growth was too small to measure accurately.

Figure 2 shows curves relating growth yields of *Streptococcus faecalis* and *Saccharomyces cerevisiae* to glucose concentration. In the case of *S. cerevisiae* it was found that the organism would grow in the absence of oxygen only when the medium was reinforced with ergosterol (cf. Andreasen & Stier, 1953); the steroid was not required for aerobic growth. The slopes of the two curves shown in Fig. 2 are identical, implying that the values of $Y_{glucose}$ for *S. faecalis* and *S. cerevisiae* under these conditions are the same. It will be noted from Fig. 2 that even when the partially defined medium was used there was always some growth of *S. faecalis* in the absence of added carbohydrate. Figure 3 shows the relationship between the growth of *Pseudomonas lindneri* and the glucose content of the medium. The slope suggests that $Y_{glucose}$ is about half that of *S. faecalis* and *S. cerevisiae*. In Fig. 4 are plotted the results of experiments with *Propionibacterium pentosaceum* grown anaerobically on glucose, glycerol and lactate. Comparing the slopes of these curves with those

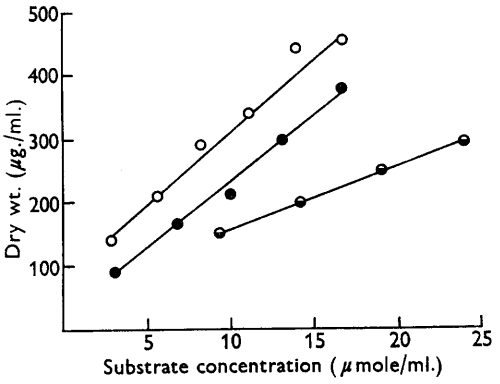

Fig. 1

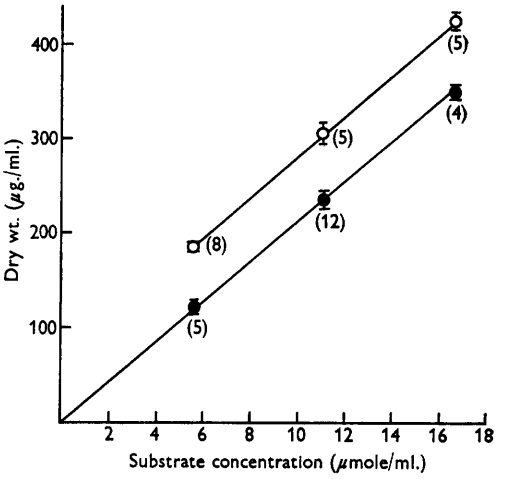

Fig. 2

Fig. 1. Growth of *Streptococcus faecalis* on glucose, ribose and arginine. Organism grown on complex medium with glucose, ribose and arginine as energy source. Incubation at 37°. Growth measured turbidimetrically and dry wt. read from a standard curve relating optical density to dry wt. ○—○ = growth on glucose; ●—● = growth on ribose; ◒—◒ = growth on arginine.

Fig. 2. Growth of *Streptococcus faecalis* and *Saccharomyces cerevisiae* on glucose. *S. faecalis* grown on partially defined medium and incubated at 37°; *S. cerevisiae* grown on medium given in text and incubated at 30°. At the end of growth cells harvested, washed and dried at 100° to constant weight. ○—○ = *S. faecalis*; ●—● = *S. cerevisiae*. Each point is the mean of the number of estimations shown in parentheses; the standard deviations from the mean are represented by the heights of the vertical bars.

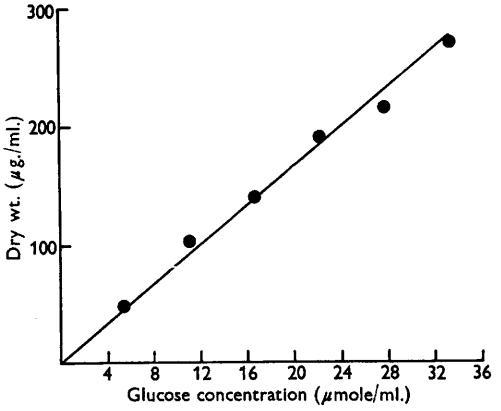

Fig. 3

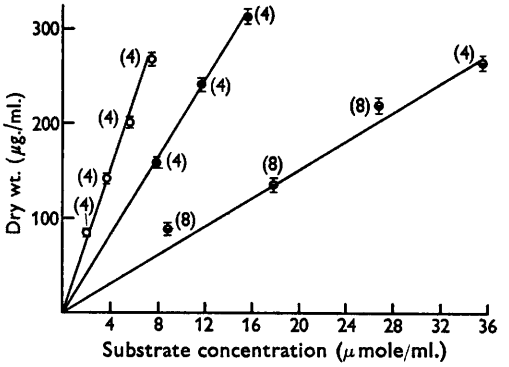

Fig. 4

Fig. 3. Growth of *Pseudomonas lindneri* on glucose. Incubation at 30°. Growth measured turbidimetrically and the dry wt. read from a standard curve relating optical density to dry wt.

Fig. 4. Growth of *Propionibacterium pentosaceum* on glucose, glycerol and lactate. Incubation at 30°. At the end of growth, organism was harvested, washed and dried at 100° to constant weight. Each point is the mean of the number of estimations shown in parentheses; the standard deviations from the mean are represented by the heights of the vertical bars. ○—○ = glucose; ●—● = glycerol; ◒—◒ = sodium lactate.

in Fig. 2 for *S. faecalis* and *S. cerevisiae* it will be seen that the value of $Y_{glucose}$ of *P. pentosaceum* is considerably greater than that of the other two organisms when grown on the same substrate, suggesting that *P. pentosaceum* obtains more energy from glucose than either *S. faecalis* or *S. cerevisiae*.

The growth of Streptococcus faecalis *on* L-*arginine*

It has already been shown that when L-arginine was added to the complex medium the yield of *Streptococcus faecalis* was increased and that the growth was a linear function of the amount of arginine added. When, however, the experiment was repeated with the partially defined medium the addition of arginine did not increase the slight growth above that obtained with the medium alone; it will be recalled that there was good growth on this medium when glucose was the substrate (see Fig. 2). Two explanations appeared possible: either the complex medium contains a factor which is essential for growth on arginine; or the inoculum contained no arginine dihydrolase, the enzyme system which is concerned with the catabolism of arginine (Slade & Slamp, 1952). This latter hypothesis was found to be untenable because organisms known to contain arginine dihydrolase failed to grow when inoculated into the partially defined medium to which only arginine had been added. It was next found that addition of arginine to the partially defined medium containing a small amount of glucose raised the yield of organism above that obtained with the medium to which only glucose had been added; but the response to arginine was irregular. Figure 5 shows the results of an experiment in which four different glucose concentrations were used, and it will be seen that a linear response to arginine was obtained only with the highest glucose concentration. However, even with the highest glucose concentration tested the results were not reproducible. These experiments suggested that whilst glucose promoted the utilization of arginine for growth, the factor which was essential for growth under these conditions disappeared from the medium during growth at a rate which varied from experiment to experiment. Since glucose was known to be utilized under these conditions it seemed possible that it was the missing factor and that the irregular results observed were due to a utilization of all the glucose in the early stages of growth. Slade & Slamp (1952) had in fact found in their experiments that arginine only began to disappear from the medium when 70% of the glucose had been utilized, and the concentration of glucose in their medium was considerably greater than that used in these experiments. To test this hypothesis *S. faecalis* was grown in continuous culture on the partially defined medium in the apparatus described by Rosenberger & Elsden (1960).

With this technique fresh medium is added continuously to the culture at a constant rate, and the effect of supplementing with arginine could thus be investigated under conditions where glucose was present at all times albeit at a low concentration. The culture was started using the partially defined medium plus glucose at a concentration of 2·4 μmole/ml. When the concentration of cells approached the expected value, the flow of medium into the culture vessel was started and a steady state was reached 18 hr. after inoculation. The culture was allowed to grow in this way for a further 24 hr. during which time the cell concentration and the pH value of the culture were measured at suitable time intervals. Arginine was then added to the medium in the reservoir to give a final concentration

of 9·5 μmole/ml.; almost immediately the cell concentration began to increase and 10 hr. after the addition of the arginine was started a new steady state was reached which was maintained for a further 30 hr. The complete protocol of the experiment is given in Table 1; Fig. 6 is a plot of the dry wt. organism/ml. against time from the eighteenth hour onward. The mean dry wt. organism/ml. during the glucose steady state was 119 μg. ml. and in the steady state in the presence of glucose + arginine 219 μg. ml. The yield of organism due to arginine was therefore 100 μg. ml. The concentration of arginine in the medium was 9·5 μmole/ml., giving a Y_{arginine} of 10·5; this agrees well with the value of 10 obtained in the experiment with the complex medium.

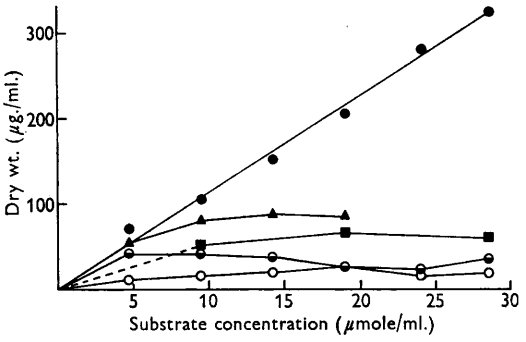

Fig. 5

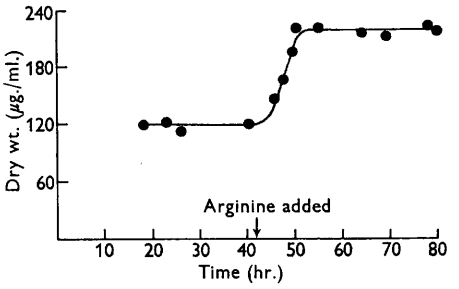

Fig. 6

Fig. 5. Effect of glucose on the growth of *Streptococcus faecalis* in partially defined medium containing arginine. Incubation at 37°. Growth measured turbidimetrically and the dry wt. read from a graph relating optical density to dry wt. ○—○ = no glucose; ◒—◒ = 0·56 μmole glucose/ml.; ■—■ = 2·78 μmole glucose/ml.; ▲—▲ = 4·17 μmole glucose/ml.; ●—● = 5·56 μmole glucose/ml.

Fig. 6. Growth of *Streptococcus faecalis* in continuous culture. The partially defined medium was used which contained initially 2·5 μmole glucose/ml. At 41·75 hr., marked by arrow, sterile arginine solution was added to the reservoir to give a final concentration of 9·5 μmole arginine/ml. Growth measured turbidimetrically and dry wt. read from a graph relating optical density to dry wt.

Assimilation of glucose by Streptococcus faecalis

In calculating the yield coefficients it was assumed that all the substrate added was catabolized. To test the validity of this assumption *Streptococcus faecalis* was grown on uniformly labelled D-(^{14}C)-glucose, using 150 ml. of the partially defined medium containing 0·1% (w/v) D-(^{14}C)-glucose, specific activity = 838 counts/min./mg. When growth ceased the optical density of the culture was measured with the Spekker absorptiometer and the organisms harvested by centrifuging at 2° in an International refrigerated centrifuge. The organisms were washed once with 100 ml. 5% (w/v) trichloroacetic acid (TCA), twice with 50 ml. 5% TCA and once with 50 ml. 0·05% (w/v) sodium lactate. This last was used as a carrier to remove traces of radioactive lactate. The precipitate so obtained was washed once with 50 ml. distilled water and suspended evenly in 100 ml. distilled water. Samples (5 ml.) were filtered on a circle of Whatman No. 40 filter paper, as described by Ormerod (1956) and the precipitate washed sequentially with 50% (w/v) aqueous acetone and acetone and, after drying under the infra-red lamp the filter papers were

prepared for counting. Since about 1·4 mg. cell residues were counted on disks of area 2·8 cm.^{2} self-absorption could be neglected. The elaborate washing procedure was used to ensure that the samples were free from non-cellular radioactive materials, particularly end products of the fermentation. To check the efficacy of the method organisms from a culture grown on glucose were suspended in the supernatant fluid obtained by centrifuging a culture grown on (^{14}C)-glucose. This

Table 1. *Growth of* Streptococcus faecalis *in continuous culture with glucose and arginine as energy sources*

Organism grown in partially defined medium containing, initially, 2·4 μmole glucose/ml. After 41·75 hr. arginine was added to the reservoir to give a final concentration of 9·5 μmole/ml. medium.

				Sample	
Time (hr.)	Flow rate (ml./hr.)	Volume (ml.)	Temperature (°C)	pH	Dry wt. organism (μg./ml.)
0	36	324	37·7	—	—
18	34·8	318	39·2	6·4	120
23	35·4	314	38·4	6·6	123
30	35·5	314	39·1	6·5	113
40·3	35·8	318	37·2	6·4	120
41·75	L-Arginine added to reservoir				
45·75	—	—	—	6·5	147
47·5	—	—	—	6·6	168
49·7	—	—	—	6·6	197
50·2	35·4	318	39·0	—	—
52·7	—	—	—	6·8	222
54·8	—	—	—	6·7	222
64·5	—	—	—	6·8	217
65	37·2	318	37·8	—	—
69·25	—	—	—	6·8	213
76·75	—	—	—	—	224
78·25	—	—	—	6·8	220
79·75	37·1	318	37·2	—	—

Table 2. *Growth of* Streptococcus faecalis *on* D-(^{14}C)-*glucose*

Organism grown in 150 ml. of partially defined medium containing 150 mg. D-(^{14}C)-glucose (Specific activity = 838 counts/min./mg. glucose). Incubation temperature = 37°. At the end of growth the dry wt. organism/ml. culture was measured turbidimetrically (Spekker Absorptiometer) and organisms harvested and prepared for radioactive assay as described in the text.

Dry wt. organism (mg.)	Glucose added (c.p.m.)	Radioactivity of organisms (c.p.m.)	Glucose incorporated (% of that added)
27·8	125,700	1,156	0·92

suspension was then put through the washing procedure and the radioactivity of the organisms measured; the activity was no greater than background. Table 2 shows the results of the experiment. The organisms produced weighed 27·8 mg. and the total radioactivity was 1156 counts/min., equivalent to 0·552 mg. glucose-carbon, or 0·92 % of the glucose added. If the carbon content of the cells is taken as 50 % of the dry wt. then the substrate supplied 4 % of the cell-carbon.

DISCUSSION

Table 3 lists the values of Y we have obtained for *Streptococcus faecalis*, *Saccharomyces cerevisiae*, *Pseudomonas lindneri* and *Propionibacterium pentosaceum*. Since the ATP yields of the catabolic reactions used by the first three of these organisms are known we also list in Table 3 the values for Y^{ATP} calculated from the various yield coefficients. The mean of the 8 values for Y^{ATP} is 10·5 (range = 8·3–12·6). Considering the fact that these organisms, *S. faecalis*, *S. cerevisiae* and *P. lindneri*, belong to quite different taxonomic groups, the agreement between the values is striking. We conclude from these results that, under the cultural conditions used, i.e. anaerobic growth on complete media with the energy source as the limiting factor, the yield of these three organism/mole of ATP is constant. From this it follows that the amount of biologically useful energy these organisms obtain per mole of energy source is more precisely expressed by the ATP yields of their

Table 3. *Growth yields of* Streptococcus faecalis, Saccharomyces cerevisiae, Pseudomonas lindneri *and* Propionibacterium pentosaceum

The values given are the means of all determinations shown in Figs. 1–4 and 6.

Organism	Medium	Substrate	ATP yield (mole ATP/ mole substrate)	$Y_{substrate}$ (g. dry wt. cells/mole substrate)	Y^{ATP} (g. dry wt. cells/mole ATP)
S. faecalis	Partially defined	Glucose	2·0	22	11
S. faecalis	Complex	Glucose	2·0	23	11·5
*S. faecalis**	Complex	Glucose	2·0	18·5	9·3
S. faecalis	Complex	Ribose	1·67	21	12·6
S. faecalis	Complex	Arginine	1·0	10	10
S. faecalis†	Partially defined	Arginine	1·0	10·5	10·5
S. cerevisiae	Partially defined	Glucose	2·0	21	10·5
P. lindneri	Complex	Glucose	1·0	8·3	8·3
P. pentosaceum	Complex	Glucose	—	37·5	—
P. pentosaceum	Complex	Glycerol	—	20	—
P. pentosaceum	Complex	DL-lactate	—	7·6	—

* = values taken from Sokatch & Gunsalus (1957).
† = results from continuous culture experiments.

respective catabolic reactions than by the standard free energy changes computed for these reactions. The operative phrase here is 'biologically useful energy' by which we mean that amount of energy made available by a catabolic process which the cell is able to use for the performance of the chemical, osmotic and mechanical work associated with growth. So far only three organisms have been studied and there is clearly a need for further investigations. The main requirement is for organisms with catabolic processes the ATP yields of which are known with certainty. That the ATP yield of a catabolic reaction rather than the free energy change is a more accurate index of the amount of biologically useful energy produced by that reaction is shown very clearly by the results with *S. cerevisiae* and *P. lindneri*. Both of these organisms convert glucose anaerobically to ethanol and CO_2 along with some minor end products. The yeast ferments glucose by the Embden–Meyerhof pathway with an ATP yield of two, whereas *P. lindneri* catabolizes glucose via 2-oxo-3-deoxy-6-phosphogluconate (the Entner–Doudoroff pathway) with an ATP

yield of one. Since the overall reaction is the same in both cases the free-energy changes will also be identical, regardless of the fact that the mechanisms involved differ markedly from one another. From this it follows that if the ATP yield is taken as the index of the amount of biologically useful energy made available to the organism then the value of $Y_{glucose}$ of *S. cerevisiae* will be twice that of *P. lindneri*; whereas, if the free energy change is the index, then $Y_{glucose}$ should be the same for both organisms. Table 3 shows that the $Y_{glucose}$ of *S. cerevisiae* is somewhat more than double that of *P. lindneri*.

Since the amount of ATP produced by a growing culture cannot be measured directly we have assumed in calculating the values for Y^{ATP} that all the substrate added to the medium was catabolized. In the case of *Streptococcus faecalis* direct measurement of the amount of glucose-carbon assimilated using (^{14}C)-glucose showed that only 0·92 % of that added to the medium was assimilated. This amount was equivalent to some 4 % of the cell carbon. The result was not surprising since the complex nutritional requirements of *S. faecalis* indicate a very low synthetic ability. It was also known from the work of Smith & Sherman (1942) that growing cultures of this organism convert 90–95 % of the glucose in the medium to lactic acid.

The nutritional requirements of the other organisms studied are simpler than those of *Streptococcus faecalis*, indicating that when they are grown upon their respective minimal media significant amounts of substrate are assimilated. When assimilation occurs, the amount of substrate catabolized cannot be taken as equal to the amount of substrate which disappears from the medium. However, these organisms were, for the purpose of our experiments, grown on complex media and we assume that, under these conditions, their synthetic powers were repressed and that they utilized the preformed amino acids and other essential cell constituents present in their respective media. The validity of this assumption has not been tested with these organisms, but it is known from work with other microbes, in particular with *Escherichia coli* that repressions of this sort occur: for a review of the available evidence see Magasanik, 1957; Roberts *et al.* 1955.

Table 3 shows that when *Streptococcus faecalis* was grown in a complex medium the value for $Y_{glucose}$ was, within experimental error, the same as that obtained when this organism was grown in the partially defined medium. The value for $Y_{arginine}$ obtained by growing *S. faecalis* in continuous culture on the partially defined medium supplemented with a small amount of glucose was 10·5, which agrees well with the value of 10 obtained by growing the organism in batch culture on a complex medium + arginine. The ratio $Y_{glucose}/Y_{arginine}$ calculated from our results = 2·19 and is approximately equal to the ratio of the ATP yields from glucose and arginine, respectively, viz. 2·0.

Fukui, Ôi, Ôbayashi & Kitahara (1957) found that growing cultures of *Streptococcus faecalis* fermented both D-ribose and L-arabinose to lactate; small amounts of formate but no acetate were also produced. Washed suspensions prepared from cultures grown on arabinose converted this pentose almost quantitatively to lactate. To account for these results they suggested that both ribose and arabinose are first converted to hexose by the pentose cycle enzyme system and the hexose so formed fermented via the Embden–Meyerhof pathway. Sokatch & Gunsalus (1957) came to a similar conclusion as a result of their study of the fermentation of gluco-

nate by *S. faecalis*. The ATP yield when pentose is fermented by this mechanism is 1·67 mole ATP/mole pentose (Elsden & Peel, 1958). The value we obtained for Y_{ribose} was 21 which gives $Y^{ATP} = 12{\cdot}6$, a value somewhat greater than those calculated from the results with arginine and glucose.

It will be seen from Table 3 that the value of $Y_{glucose}$ for *Propionibacterium pentosaceum* is approximately twice that of *Streptococcus faecalis* and *Saccharomyces cerevisiae* and four times that of *Pseudomonas lindneri*. If our conclusion that the growth of an organism is a function of the ATP yield of its catabolic processes is correct then it follows that *P. pentosaceum* synthesizes twice as much ATP per mole of glucose as either *S. faecalis* or *S. cerevisiae*, that is to say, the ATP yield from the fermentation of glucose by *P. pentosaceum* is 4 mole/mole glucose. The origin of this extra ATP is a matter of some interest. The fermentation of glucose is approximately described by reaction:

$$1{\cdot}5 \text{ glucose} \rightarrow 2 \text{ propionate} + \text{acetate} + 1CO_2.$$

There is evidence which suggests that the Embden–Meyerhof mechanism plays an important part in the fermentation of glucose by *P. pentosaceum* (Wood, Stjernholm & Leaver, 1955) and that pyruvate is the precursor of the end-products propionate, acetate and CO_2. If this is the case it follows that the conversion of 1·5 mole glucose to three mole pyruvate will yield 3 mole ATP. But the value for $Y_{glucose}$ requires the formation of 6 mole ATP from 1·5 mole glucose, of which only three have so far been accounted for. The formation of acetate from pyruvate should give 1 mole ATP/mole acetate and, if this is correct, we are forced to conclude that the remaining 2 mole ATP arise from the reactions concerned with the formation of propionate. In other words, the formation of 1 mole of propionate is associated with the formation of one mole of ATP. It will be possible to test this prediction once the individual reactions in the synthesis of propionate have been defined and the enzymes responsible isolated.

This work was in part supported by grants from the Rockefeller Foundation and the Kellogg Foundation to whom we express our thanks. One of us (T.B.) was in receipt of a Research Studentship from the Agricultural Research Council. Our thanks are also due to Dr R. F. Rosenberger for his help with the continuous culture experiments.

REFERENCES

AKAMATSU, S. & SEKINE, T. (1951). Hydrolysis of arginine by *Streptococcus faecalis*. *J. Biochem.* (*Japan*), **38**, 349.

ANDREASEN, A. A. & STIER, J. J. B. (1953). Anaerobic nutrition of *Saccharomyces cerevisiae*. I. Ergosterol requirement for growth in a defined medium. *J. cell. comp. Physiol.* **41**, 23.

BAUCHOP, T. (1958). Observations on some molar growth yields of bacteria and yeasts. *J. gen. Microbiol.* **18**, vii.

BURTON, R. M. (1957). In *Methods of Enzymology*, vol. 3, 246. Ed. S. R. Colowick & N. O. Kaplan. New York: Academic Press Inc.

DEMOSS, R. D., BARD, R. C. & GUNSALUS, I. C. (1951). The mechanism of the heterolactic fermentation: a new route of ethanol formation. *J. Bact.* **62**, 499.

DELWICHE, E. A. (1950). A biotin function in succinic decarboxylation by *Propionibacterium pentosaceum*. *J. Bact.* **59**, 439.

ELSDEN, S. R. & GIBSON, Q. H. (1954). The estimation of lactic acid using ceric sulphate. *Biochem. J.* **58**, 154.

ELSDEN, S. R. & PEEL, J. L. (1958). The metabolism of carbohydrates and related compounds. *Annu. Rev. Microbiol.* **12**, 145.

FUKUI, S., ÔI, A., ÔBAYASHI, A. & KITAHARA, K. (1957). Studies on the pentose metabolism by microorganisms. 1. A new type-lactic acid fermentation of pentose by lactic acid bacteria. *J. gen. appl. Microbiol.* **3**, 258.

GIBBS, M. & DEMOSS, R. D. (1954). Anaerobic dissimilation of C^{14} labelled glucose and fructose by *Pseudomonas lindneri*. *J. biol. Chem.* **207**, 689.

GUNSALUS, I. C. & GIBBS, M. (1952). The heterolactic fermentation. II. Position of C^{14} in the products of glucose dissimilation by *Leuconostoc mesenteroides*. *J. biol. Chem.* **194**, 871.

HEATH, E. C., HURWITZ, J., HORECKER, B. L. & GINSBERG, A. (1958). Pentose fermentation by *Lactobacillus plantarum*. I. The cleavage xylulose-5-phosphate by phosphoketolase. *J. biol. Chem.* **131**, 1009.

HERBERT, D. (1958). Some principles of continuous culture. In *Recent Progress in Microbiology*. Ed. G. Tunevall. Symp. 7th Int. Congr. Microbiol. p. 381.

HURWITZ, J. (1958). Pentose phosphate cleavage by *Leuconostoc mesenteroides*. *Biochim. biophys. Acta*, **28**, 599.

KNIVETT, V. A. (1954*a*). Phosphorylation coupled with anaerobic breakdown of citrulline. *Biochem. J.* **56**, 602.

KNIVETT, V. A. (1954*b*). The effect of arsenate on bacterial citrulline breakdown. *Biochem. J.* **56**, 606.

MAGASANIK, B. (1957). Nutrition of bacteria and fungi. *Annu. Rev. Microbiol.* **11**, 221.

MONOD, J. (1942). *Recherches sur la croissance des cultures bacterienne*. Paris: Herman et Cie.

O'DEA, J. F. & GIBBONS, R. A. (1953). The estimation of small amounts of formaldehyde liberated during the oxidation of carbohydrate and other substances with periodate. *Biochem. J.* **55**, 580.

ORMEROD, J. G. (1956). The use of radioactive carbon dioxide in the measurement of carbon dioxide fixation in *Rhodospirillum rubrum*. *Biochem. J.* **64**, 373.

ROBERTS, R. B., COWIE, D. B., ABELSON, P. H., BOLTON, E. & BRITTEN, R. (1955). In *Studies of Biosynthesis in* Escherichia coli. *Publ. Carneg. Instn*, no. 602.

ROSENBERGER, R. F. & ELSDEN, S. R. (1960). The yields of *Streptococcus faecalis* grown in continuous culture. *J. gen. Microbiol.* **22**, 727.

SLADE, H. D. & SLAMP, W. C. (1952). The formation of arginine dihydrolase by streptococci and some properties of the enzyme system. *J. Bact.* **64**, 455.

SMITH, P. A. & SHERMAN, J. H. (1942). The lactic acid fermentation of Streptococci. *J. Bact.* **43**, 725.

SOKATCH, J. T. & GUNSALUS, I. C. (1957). Aldonic acid metabolism. I. Pathway of carbon in an inducible gluconate fermentation by *Streptococcus faecalis*. *J. Bact.* **73**, 452.

WHITE, J. & MUNNS, D. J. (1951). The effect of aeration and other factors on yeast growth and fermentation. *Wallerstein Lab. Comm.* **14**, 199.

WOOD, H. G., STJERNHOLM, R. & LEAVER, F. W. (1955). The metabolism of labelled glucose by the propionic acid bacteria. *J. Bact.* **70**, 510.