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Glucose Catabolism by *Spirochaeta thermophila* RI 19.B1

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Spirochaeta thermophila **RI 19.B1 (DSM 6192) fermented glucose to lactate, acetate, CO_2, and H_2 with concomitant formation of cell material. The cell dry mass yield was 20.0 g/mol of glucose. From the fermentation balance data and knowledge of the fermentation pathway, a Y_{ATP} of 9.22 g of dry mass per mol of ATP was calculated for pH-uncontrolled batch-culture growth on glucose in a mineral medium. Measurement of enzyme activities in glucose-grown cells revealed that glucose was taken up by a permease and then subjected to ATP-dependent phosphorylation by a hexokinase. Glucose-6-phosphate was further metabolized to pyruvate through the Embden-Meyerhof-Parnas pathway. The phosphoryl donor for phosphofructokinase activity was PP_i rather than ATP. This was also found for the type strain of *S. thermophila*, Z-1203 (DSM 6578). PP_i was probably formed by pyrophosphoroclastic cleavage of ATP, with recovery of the resultant AMP by the activity of adenylate kinase. All other measured kinase activities utilized ATP as the phosphoryl donor. Pyruvate was further metabolized to acetyl coenzyme A with concomitant production of H_2 and CO_2 by pyruvate synthase. Lactate was also produced from pyruvate by a fructose-1,6-diphosphate-insensitive lactate dehydrogenase. Evidence was obtained for the transfer of reducing equivalents from the glycolytic pathway to hydrogenase to produce H_2. No formate dehydrogenase or significant ethanol-producing enzyme activities were detected.**

Studies on carbohydrate catabolism of thermophilic eubacterial anaerobes have been carried out on only a limited range of bacterial types. These have been mainly *Clostridium* spp. and the related (4) *Thermoanaerobium* spp. (5, 18, 20–22, 30). These organisms all use the Embden-Meyerhof-Parnas pathway of glucose fermentation (40). This pathway is also used by the thermophilic *Acetomicrobium* spp. (35, 39).

Two strains of extremely thermophilic spirochaetes have been recently isolated from island hot springs with seawater-modified thermal waters. Strain Z-1203, isolated from the Kuril Islands (2), has been designated the type strain of a new species, *Spirochaeta thermophila* (1), and strain RI 19.B1, isolated from the Kermadec Islands (33), has been assigned to the same species (1). This species is able to grow at temperatures as high as 73°C and is able to grow on a number of carbohydrates, including cellulose and glucose, on a mineral medium. In the study reported in this communication, we set out to determine the pathway of glucose fermentation in *S. thermophila* RI 19.B1 and to apply this knowledge to fermentation balance and growth yield data to estimate the net ATP yield.

MATERIALS AND METHODS

Bacterial strains. *S. thermophila* RI 19.B1 (DSM 6192) was isolated in an earlier study (33). The organism was grown in sulfide-reduced bicarbonate-buffered mineral medium as previously described (33). *S. thermophila* Z-1203 (DSM 6578) (1, 2) was obtained from the Institute of Microbiology, Academy of Sciences of the former USSR, and supplied as a fresh cell pellet by F. A. Rainey (University of Waikato, Hamilton, New Zealand). This strain was grown in the same medium with NaCl and $MgCl_2 \cdot 6H_2O$ increased to 15 g and 2.3 g/liter, respectively, and with the addition of 1.0 g of yeast extract per liter.

Enzyme assays. Crude cell extracts were prepared from cells washed and resuspended in 100 mM Tris (pH 7.5 with HCl) as described by Janssen and Harfoot (16). Protein was estimated by a micromethod adaptation of the Lowry et al. method (34). Enzymes and biochemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.). Gases were purchased from New Zealand Industrial Gases (Wellington, New Zealand). Glyceraldehyde-3-phosphate was prepared from monobarium glyceraldehyde-3-phosphate diacetyl by treatment with Dowex-50W hydrogen form (100-200 mesh) as directed by the supplier (Sigma).

Acetate kinase assays were carried out at 50°C in stoppered N_2-flushed test tubes. At each 5-min time point three assays (and appropriate controls) were halted and put on ice. The assay was essentially method I of Nishimura and Griffith (25), except that succinate was omitted and 5 mM ATP (EC 2.7.2.1) or 5 mM PP_i (EC 2.7.2.12) was used as the phosphoryl donor. The activity measured is expressed as micromoles of hydroxamate derivative formed per minute per milligram of crude cell extract protein.

All other enzyme activities were measured in 1-ml volumes by using continuous spectrophotometric assays (13) carried out at 50°C. Under these conditions the mesophilic linking enzymes remained active to produce linear rates of measured enzyme activity for 2 to 10 min. Assays containing methylviologen (MV) were first slightly reduced with a few crystals of sodium dithionite and then gassed as appropriate for the assay with H_2 or N_2, stoppered, and vigorously shaken to dissolve the gas in the buffer. Oxidation and reduction of pyridine nucleotides were followed at 340 nm (6). Other electron acceptors were measured as described by Möller-Zinkhan and Thauer (24). Enzyme activities are expressed as micromoles of substrate transformed per minute per milligram of crude cell extract protein. The reduction of 2 μmol of MV was defined as the oxidation of 1 μmol of substrate.

The following enzymes were assayed by using reaction

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mixtures as described by Lamed and Zeikus (21) with the following modifications: fructose-1,6-diphosphate aldolase (EC 4.1.2.13) omitting cysteine; glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12); glucose-6-phosphate dehydrogenase with NAD or NADP (EC 1.1.1.49) with 2.5 mM $MgCl_2$ and 2 mM dithiothreitol; gluconate-6-phosphate dehydrogenase with NAD (EC 1.1.1.43) or NADP (EC 1.1.1.43/44) and with 2.5 mM $MgCl_2$ and 2 mM dithiothreitol; lactate dehydrogenase (EC 1.1.1.27/28) omitting fructose-1,6-diphosphate for fructose-1,6-diphosphate-insensitive activity; pyruvate synthase adding 2 mM reduced glutathione and using 2 mM MV (EC 1.2.7.1), NAD^+, $NADP^+$, FMN^+, or FAD^+; pyruvate kinase with 5 mM ADP (EC 2.7.1.40) or KH_2PO_4. Glucose dehydrogenase with NAD (EC 1.1.1.118) or NADP (EC 1.1.1.119) was assayed by substituting glucose for glucose-6-phosphate in the assay for glucose-6-phosphate dehydrogenase.

The following enzymes were assayed by using the reaction mixtures of Patni and Alexander (30) with the following modifications: phosphoglucose isomerase (EC 5.3.1.9) with 2 U of glucose-6-phosphate dehrogenase; phosphoglycerate mutase (EC 5.4.2.1) with 2 U each of enolase, pyruvate kinase, and lactate dehydrogenase; enolase (EC 4.2.1.11) with 2 U each of pyruvate kinase and lactate dehydrogenase; 3-phosphoglycerate kinase with 2 U of glyceraldehyde-3-phosphate dehydrogenase and 3.2 mM ATP (EC 2.7.2.3) or PP_i.

Ethanol dehydrogenase (EC 1.1.1.12) was assayed by using method A of Lamed and Zeikus (22). Pyruvate decarboxylase (EC 4.1.1.1) was assayed by a modification of the method of Bergmeyer et al. (7), with 200 mM citrate buffer (pH 6.0), 30 mM sodium pyruvate, 0.2 mM NADH, and 2 U of ethanol dehydrogenase. Phosphotransacetylase (EC 2.3.1.8) was assayed as described by Bergmeyer et al. (7). Hydrogenase (EC 1.18.99.1) was assayed by the method of Badziong and Thauer (3). Hydrogen dehydrogenase (EC 1.12.1.2) was assayed by a modification of this method, replacing MV with 1.2 mM NAD^+ and adding 2 mM dithiothreitol. Phosphofructokinase was assayed by a modification of method B of O'Brien et al. (26) with the following reaction mixture: 60 mM imidazole, 3.5 mM $MgCl_2$, 1.5 mM fructose-6-phosphate, 0.2 mM NADH, 0.35 U aldolase, 5 U of α-glycerol-3-phosphate dehydrogenase, 50 U of triosephosphate isomerase, and 1 mM ATP (EC 2.7.1.11), PP_i (EC 2.7.1.90), thiamine pyrophosphate, phosphoenolpyruvate, or KH_2PO_4 (pH 7.4 with HCl).

Hexokinase was assayed with the following reaction mixtures: 100 mM Tris, 2.5 mM $MgCl_2$, 12.5 mM glucose, 5 mM dithiothreitol, 2 U of glucose-6-phosphate dehydrogenase, 0.5 mM $NADP^+$, and 2 mM ATP (EC 2.7.1.1), phosphoenolpyruvate, or PP_i (pH 7.5 with HCl).

Triosephosphate isomerase (EC 5.3.1.1) was measured with the following reaction mixture: 250 mM triethanolamine, 4 mM glyceraldehyde-3-phosphate, 1.25 mM NADH, and 2 U of α-glycerol-3-phosphate dehydrogenase (pH 7.6 with HCl).

Acetaldehyde dehydrogenase (coenzyme A [CoA] acetylating) (EC 1.2.1.10) was measured with the following reaction mixture: 100 mM Tris, 2 mM dithiothreitol, 0.1 mM CoA, 2 mM reduced glutathione, 7 mM arsenate, 10 mM acetaldehyde, 0.5 U of phosphotransacetylase, and 2 mM NAD^+ or $NADP^+$ (pH 7.8 with acetic acid).

Formate dehydrogenase was assayed with the following reaction mixture: 50 mM 3-(*N*-morpholino)propanesulfonic acid, 20 mM sodium formate, 2 mM dithiothreitol, 5 mM $MgCl_2$, and 1.2 mM NAD^+ (EC 1.2.1.2), 1.2 mM $NADP^+$ (EC 1.2.1.43), or 5 mM MV (pH 7.5 with KOH).

Ferredoxin:NAD oxidoreductase (EC 1.8.1.1/3/4) was assayed by monitoring MV reduction with the following reaction mixture: 100 mM Tris, 5 mM MV, and 0.25 mM NADH (pH 8.8 with HCl).

Adenylate kinase (EC 2.7.4.3), ATP pyrophosphatase (EC 3.6.1.8), and ATP:orthophosphate phosphotransferase were assayed with the following reaction mixture: 100 mM Tris, 5 mM $MgCl_2$, 2 mM dithiothreitol, 10 mM glucose, 2 U of hexokinase, 2 U of glucose-6-phosphate dehydrogenase, and 2 mM $NADP^+$ (pH 7.4 with HCl). Adenylate kinase assays also contained 5 mM ADP. ATP pyrophosphatase assays also contained 5 mM AMP and 1 mM PP_i. ATP:orthophosphate phosphotransferase assays also contained 5 mM ADP and 1 mM PP_i.

Other methods. The methods used in the growth experiments and yield studies were described earlier (33). Fermentation end products were analyzed by high-pressure liquid chromatography (HPLC) (28) and by gas chromatography with a thermal conductivity detector and gas-liquid chromatography with a flame ionization detector (29). In addition, the presence of further metabolic end products was checked by using the *p*-hydroxybenzoic acid hydrazine assay for reducing sugars (23), the anthrone assay for carbohydrates (11), the cuprizone assay for copper complexing compounds such as amino acids (15), and dinitrophenol hydrazine for aldehydes and ketones (17).

Glucose phosphorylation by decryptified cells was measured by using the assay for glucokinase described above but with added decryptified cells prepared by the method of Kornberg and Reeves (19) with a toluene-ethanol mixture (1:9) as the permeabilizing agent so that the final toluene concentration was 1 μl/ml.

RESULTS

Fermentation balances. Fermentation end products were analyzed by using a variety of techniques, including HPLC, gas chromatography, and a number of colorimetric methods. When glucose was exhausted, a residual level of carbohydrate could be detected with colorimetric methods and an unidentified peak was present on HPLC chromatograms. This unidentified peak was also formed in uninoculated media containing glucose, and the rate of production could be increased by incubation of uninoculated media at higher temperatures. The amount of glucose utilized was therefore corrected for this chemical transformation. The metabolic end products of glucose metabolism were lactate, acetate, CO_2, and H_2, in agreement with the earlier study (33). The means of data from three independent cultures are given in Table 1. The oxidation/reduction ratio is low, indicating an excess of reducing equivalents, but this value is strongly influenced by the amount of CO_2 evolved, which could only be estimated. Measurement of CO_2 was not possible because of the use of a bicarbonate buffer. The balances of carbon and available H were good, indicating that all major end products were identified.

Glucose uptake. Toluene-permeabilized cells were assayed for their ability to phosphorylate glucose coupled to either ATP or phosphoenolpyruvate as the phosphoryl donor (Table 2). The addition of ATP resulted in measurable rates of glucose transformation, whereas the addition of phosphoenolpyruvate did not, indicating a hexokinase activity rather than a phosphoenolpyruvate-phosphotransferase for glucose uptake. This activity was confirmed by the measurement of

TABLE 1. Fermentation end products and balances of glucose fermentation by *S. thermophila* RI 19.B1[a]

Substrate or product (mol/liter)	Carbon balance		Hydrogen balance	
	mol/100 mol of substrate	mol of carbon	Available H (mol/100 mol of substrate)	Redox value (mol/100 mol of substrate)
Glucose (8.67)	100.0	600.0	2,400.0	
Lactate (4.46)	51.4	154.2	616.8	
Acetate (10.18)	117.4	234.8	939.2	
H_2 (25.57)	294.9		589.8	−294.9
CO_2[b] (10.18)	117.4	117.4		+238.8
Cells[c] (1.14)	13.1	78.6	376.0	−30.8
Total		585.0	2,521.8	

[a] Calculations were made as described by Gottschalk (9), assuming $<C_6H_{10.84}N_{1.4}O_{3.07}>$ as the empirical formula for cell material (37). The carbon balance (substrate carbon recovered in products was 97.5%, and the available H balance was 105.1%. The oxidation/reduction ratio was 0.73.

[b] CO_2 could not be measured because it was used as a buffer; instead, CO_2 production was calculated by assuming the formation of 1 mol of CO_2 per mol of acetate, in accord with the measured pyruvate synthase activity (see Table 4).

[c] Calculated from a mean dry weight cell yield of 173.4 mg/liter.

hexokinase activity in crude cell extracts. ATP acted as a phosphoryl donor, but phosphoenolpyruvate or PP_i did not (Table 2).

Pathway of glucose catabolism. Enzyme activities were measured in crude cell extracts of glucose-grown cells of strain RI 19.B1. The enzyme activities measured showed that a typical Embden-Meyerhof-Parnas pathway was active (Table 3). Two interesting activities were detected: a very high activity of triose phosphate isomerase (EC 5.3.1.1) and a PP_i-dependent phosphofructokinase (EC 2.7.1.90) instead of the more usual ATP-dependent phosphofructokinase (EC 2.7.1.11).

The phosphoryl donor for phosphofructokinase activity was also assayed in glucose-grown cells of strain Z-1203. This strain also had a PP_i-dependent phosphofructokinase activity (0.30 μmol/min per mg of protein), whereas the addition ATP produced a much lower rate of fructose-1,6-diphosphate formation (0.0033 μmol/min per mg of protein).

Low activities of ATP pyrophosphatase (EC 3.6.1.8) and good activities of adenylate kinase (EC 2.7.4.3) were measurable in crude cell extracts of strain RI 19.B1 (Table 3).

End product formation. Measurement of enzyme activities leading from pyruvate to end products (Table 4) showed that a pyruvate synthase (EC 1.2.7.1) was active. This activity was dependent on the addition of CoA. In the enzyme assays, MV was used as an artificial electron acceptor, but in vivo the acceptor is presumed to be ferredoxin. Lactate dehydrogenase, phosphotransacetylase, and acetate kinase activities were also detected, in agreement with the end products formed. Enzymes of ethanol-forming pathways were scarcely detectable.

Hydrogenase (EC 1.18.99.1) activity (with MV as a ferredoxin substitute) and ferredoxin:NAD oxidoreductase (EC 1.8.1.1/3/4) activity (again with MV as a substitute) were also measurable, indicating enzyme steps for the production of hydrogen from reduced electron-carrying cofactors. No formate dehydrogenase (EC 1.2.1.2/43) activities were detected.

TABLE 2. Glucose uptake in decryptified cells and crude cell extracts of glucose-grown *S. thermophila* RI 19.B1

Assay or enzyme	Sp act (μmol/min per mg of protein)
Glucose uptake by decryptified cells with:	
ATP	0.15
PEP[a]	0.013
Glucose phosphorylation by crude cell extracts	
Hexokinase + ATP (EC 2.7.1.1)	0.52
Hexokinase + PP_i	<0.0001
PEP-phosphotransferase system	<0.0001

[a] PEP, phosphoenolpyruvate.

TABLE 3. Glycolytic and related enzyme activities in crude cell extracts of glucose-grown *S. thermophila* RI 19.B1

Enzyme activity	EC no.	Sp act (μmol/min per mg of protein)
Phosphoglucose isomerase	5.3.1.9	1.29
Phosphofructokinase		
+ ATP	2.7.1.11	0.0033
+ TPP[a]		0.021
+ PEP[b]		<0.0001
+ PP_i	2.7.1.90	4.63
Fructose-1,6-diphosphate aldolase	4.1.2.13	1.87
Triosephosphate isomerase	5.3.1.1	34.3
Glyceraldehyde-3-phosphate dehydrogenase		
+ NAD	1.2.1.12	3.10
+ NADP	1.2.1.9	<0.0001
3-Phosphoglycerate kinase		
+ ATP	2.7.2.3	4.88
+ PP_i		<0.0001
Phosphoglycerate mutase	5.4.2.1	7.06
Enolase	4.2.1.11	1.11
Pyruvate kinase		
+ ATP	2.7.1.40	0.96
+ PP_i		<0.0001
Glucose-6-phosphate dehydrogenase		
+ NAD	1.1.1.49	0.0003
+ NADP	1.1.1.49	0.046
Gluconate-6-phosphate dehydrogenase		
+ NAD	1.1.1.43	0.0001
+ NADP	1.1.1.43/44	0.12
Glucose dehydrogenase		
+ NAD	1.1.1.118	<0.0001
+ NADP	1.1.1.119	0.0024
ATP pyrophosphatase	3.6.1.8	0.0075
Adenylate kinase	2.7.4.3	0.13
ATP:P phosphotransferase		<0.0001

[a] TTP, thiamine pyrophosphate.

[b] PEP, phosphoenolpyruvate.

DISCUSSION

Measurements of enzyme activities showed that glucose uptake was mediated by a permease and then ATP-dependent phosphorylation rather than by a phosphoenolpyruvate-dependent phosphotransferase uptake system. This seems to be the case in glycolytic thermophiles investigated to date (20, 21, 30). The enzyme activities measured suggested that the pathway of glucose fermentation was a typical Embden-Meyerhof-Parnas pathway, with the exception that the phos-

TABLE 4. Enzyme activities involved in end product formation in crude cell extracts of glucose-grown *S. thermophila* RI 19.B1

Enzyme activity	EC no.	Sp act (μmol/min per mg of protein)
Lactate dehydrogenase		
+ FDP[a]	1.1.1.27/28	0.20
− FDP	1.1.1.27/28	0.20
Pyruvate synthase		
+ MV	1.2.7.1	0.29
+ NAD		0.0076
+ NADP		0.016
+ FMN		<0.0001
+ FAD		0.0009
Phosphotransacetylase	2.3.1.8	1.36
Acetate kinase		
+ ATP	2.7.2.1	2.07
+ PP_i	2.7.2.12	0.088
Ethanol dehydrogenase		
+ NAD	1.1.1.12	<0.0001
+ NADP	1.1.1.12	0.0042
Acetaldehyde dehydrogenase (CoA acetylating)		
+ NAD	1.2.1.10	0.0024
+ NADP	1.2.1.10	0.0018
Formate dehydrogenase		
+ NAD	1.2.1.2	<0.0001
+ NADP	1.2.1.43	<0.0001
+ MV		<0.0001
Pyruvate decarboxylase	4.1.1.1	<0.0001
Hydrogenase		
+ NAD	1.12.1.2	<0.0001
+ MV	1.18.99.1	0.066
Ferredoxin:NAD oxidoreductase	1.8.1.1/3/4	0.58

[a] FDP, fructose-1,6-diphosphate.

phorylation of fructose-6-phosphate was mediated by a PP_i-dependent phosphofructokinase (EC 2.7.1.90) instead of by the more usual ATP-dependent phosphofructokinase (EC 2.7.1.11). PP_i-dependent phosphofructokinase activity has been detected in a number of other microorganisms, for example, *Anaeroplasma* spp. (31), *Propionibacterium shermanii* (26), some methylotrophs (38), and some other bacteria (32). This activity was also found to be the phosphoryl donor to fructose-6-phosphate in *S. thermophila* Z-1203 isolated from Shiashkotan Island, Kamchatka, in the former USSR. Thus this enzyme activity appears to be a characteristic of this species, but it has not been reported to date to occur in other *Spirochaeta* spp.

A possible source of PP_i for the phosphorylation reaction is suggested by measured enzyme activities. A low level of ATP pyrophosphatase activity (EC 3.6.1.8) produces PP_i and AMP. AMP can then be converted to ADP at the expense of ATP by adenylate kinase (EC 2.7.4.3). The low level of ATP pyrophosphatase measured may be explainable by an equilibrium that does not favor the direction of measurement (AMP to ATP) or instability of the enzyme under assay conditions or by implicating adenylate kinase activity in removing AMP to drive the reaction of PP_i production. This may represent an important regulatory mechanism in the control of catabolism in this organism.

Glucose fermentation by the Embden-Meyerhof-Parnas pathway has been reported in other *Spirochaeta* spp. and related bacteria (8, 10, 12, 27). In these organisms the resultant pyruvate is further metabolized to acetyl-CoA with concomitant ferridoxin reduction (8, 12). A CoA-dependent pyruvate:MV oxidoreductase (pyruvate synthase) activity was measurable in crude cell extracts of *S. thermophila* RI 19.B1, presumably with ferridoxin under physiological conditions.

The measured enzyme activities confirm the end product formation pattern. Lactate dehydrogenase (not stimulated by fructose-1,6-diphosphate) producing lactate, pyruvate synthase producing CO_2 and acetyl-CoA, and then phosphotransacetylase and acetate kinase producing acetate confirm the carbon-containing end products detected. Formate dehydrogenase and ethanol-forming enzyme activities were not significant.

Pyruvate synthase activity produces reduced ferredoxin, which can then donate electrons to reduce protons to form H_2. The reduced pyridine nucleotide cofactors (NADH) produced from the Embden-Meyerhof-Parnas pathway (at the step of glyceraldehyde-3-phosphate dehydrogenase activity) could apparently reduce MV and thus suggest a pathway for transfer of reducing potential to hydrogenase via ferredoxin or another carrier. This possibility is confirmed by the recovery as H_2 of all reducing potential not transferred to lactate or cell material.

The reduction of ferredoxin by NADH normally only proceeds at a low partial pressure of H_2 (9). However, in a number of anaerobes (for example, *Acetomicrobium* spp.) this reaction appears to proceed against a high partial pressure of H_2 (35, 39). This also occurs in *S. thermophila* RI 19.B1. The mean final H_2 partial pressure in the culture headspace in balance experiments with an initial 10 mM glucose concentration was 99 kPa (14), corresponding to a dissolved H_2 concentration of 0.53 mM at 65°C (calculated from data in reference 36). Under these conditions the reduction of ferredoxin is endergonic and theoretically requires an energy input; for example, some type of reverse electron transport (9). In the present study, cell extracts of glucose-grown *S. thermophila* RI 19.B1 were able to reduce MV with NADH as the electron donor. This activity indicates electron transfer to ferredoxin from the glycolytic pathway and thus to hydrogenase.

The purple pigmentation noted in the earlier study characterizing *S. thermophila* RI 19.B1 (33) was shown to be resazurin (14). The end product spectrum and ratios were not changed when cultures were grown without resazurin (producing unpigmented cells).

When the fermentation balance data and measured enzyme activities are applied to obtain a theoretical ATP yield, the following calculations can be made. From 8.67 mmol of glucose, 8.67 mmol of fructose-1,6-diphosphate is produced at the expense of 26.01 mmol of ATP. This is 3 mol of ATP per mol of glucose, rather than the 2 mol of ATP per mol of glucose that would be expected if a normal ATP-dependent phosphofructokinase activity were present. First 1 mol of ATP is expended per mol of glucose in the hexokinase reaction. One ATP is expended per mol of PP_i produced, i.e., per mol fructose-6-phosphate phosphorylated to fructose-1,6-diphosphate; then a further ATP is expended to recover the AMP produced by the activity of adenylate kinase. Further activity of the Embden-Meyerhof-Parnas pathway then results in the transformation of the fructose-1,6-phosphate to 17.34 mmol of glyceraldehyde-3-phosphate, which is then transformed to 17.34 mmol of pyruvate with the concomitant formation of 34.68 mmol of ATP by the activity of the enzymes 3-phosphoglcerate kinase and pyruvate kinase. Substrate-level phosphorylation then adds an additional 10.18 mmol ATP, estimated from the amount of acetate detected as an end product. Thus a net total of 18.85

mol of ATP was produced from 8.67 mmol of glucose, a yield of 2.17 mol/mol. The cell dry mass produced was 20.0 g/mol of glucose, and thus the Y_{ATP} can be calculated at 9.22 g of dry mass per mol of ATP.

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