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Growth Yields of Bacteria on Selected Organic Compounds

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Cell yields were determined for two bacterial soil isolants grown aerobically in minimal media on a variety of synthetic organic compounds. 1-Dodecanol, benzoic acid, phenylacetic acid, phenylglyoxylic acid, and diethylene, triethylene, and tetraethylene glycols were tested. Two "biochemicals," succinate and acetate, were also tested for comparison. Yields were calculated on the basis of grams of cells obtained per mole of substrate utilized, gram atom of carbon utilized, mole of oxygen consumed, and equivalent of "available electrons" in the substrates. This latter value appears to be nearly constant at 3 g of cells per equivalent of "available electrons." Yields predicted on this basis for other bacteria and for yeasts on other substrates are in fair agreement with reported values.

Some years ago, Monod (13) described the reproducibility of growth yields of cells grown aerobically in minimal media and the dependence of these yields on the amount of carbohydrate supplied to the culture. Bauchop and Elsden (1), working with organisms grown anaerobically in media richly supplemented with precursors of cellular material, demonstrated that 10.5 g of cells resulted for each mole of adenosine triphosphate generated when glucose or arginine was fermented solely as an energy source. Storck and Stanier (18) have considered various aspects of growth of aerobes, and possible energy relationships involved, when cells are grown aerobically on citric acid cycle intermediates or certain aromatic acids as sole sources of carbon and energy.

There has recently been a resurgence of interest in the determination of growth yields of microorganisms on various substrates. For example, Johnson (8) has considered yields resulting from aerobic growth of bacterial and fungal species on hydrocarbons with an orientation toward production of nutritive materials, i.e., food or fodder supplements from noncrop chemicals.

Studies in our laboratory have been based on the premise that it may be possible, and indeed desirable, to transform waste chemicals which are (or have the potential of becoming) pollutants into protein or other nutrients, by growing bacteria on them. The validity of such a premise is substantiated by the inclusion of considerations of microbial transformations of pollutants to usable materials, as items for immediate emphasis by the Subcommittee on Use and Management of Biological Resources, U.S. National Committee for the International Biological Program (National Research Council. 1967. Program statement of the Subcommittee on Use and Management of Biological Resources).

Our studies were directed toward finding the most significant parameters that relate yields of dry weight of cells to quantities of synthetic organic substrates utilized as sole sources of carbon and energy during aerobic growth in minimal, mineral media. We have attempted to identify a common basis for predicting yields from various substrates. Portions of this work have been reported elsewhere (16, 17).

MATERIALS AND METHODS

Bacteria. Two soil isolants were used in these studies. *Pseudomonas* C12B, isolated from river bank soil by enrichment with detergents (15), was used to determine cell yields from benzoic acid, phenylglyoxylic acid, phenylacetic acid, 1-dodecanol, succinate, acetate, and an equimolar mixture of succinate and acetate. Cultures of this organism were maintained on nutrient agar slants at room temperature with monthly transfers.

The second isolant, designated TEG-5, was isolated from soil by enrichment with triethylene glycol (5), and was used to determine cell yields from diethylene, triethylene, and tetraethylene glycols. Cultures of TEG-5 were maintained on 0.25% tetraethylene glycol-basal salts-agar slants at room temperature with monthly transfers.

Preparation of inocula. Cells were adapted to the test substrates by three transfers into a basal salts medium (15) containing the compound of interest at a concentration of 0.25%, w/v, with incubation at 30 C on a rotary shaker.

Adapted cells were harvested by centrifugation

(37,000 × *g* for 30 min) of late-log-phase cultures, and washed twice with sterile basal salts medium to remove soluble substrates. In the case of cells grown on 1-dodecanol, harvested cells were resuspended in a volume of sterile basal salts equal to that of the original culture and incubated an additional 8 hr to exhaust residual dodecanol. (Because of its insolubility in water, 1-dodecanol adheres to the cells and is removed with difficulty only after repeated washing.) The substrate-free cells were then reharvested and washed twice with sterile basal salts.

Washed adapted cells from the various cultures were resuspended in the sterile mineral medium to an optical density of 0.65 at 420 mμ. Portions of 0.5 ml each were used as inocula.

Chemicals. 1-Dodecanol was purified from Alfol 12 (Continental Oil Co., Ponca City, Okla.) by fractional distillation to a purity estimated by gas-liquid chromatography (GLC) to be 99.9%. Benzoic acid sodium salt (Eastman Organic Chemicals, Rochester, N.Y.), and phenylacetic and phenylglyoxylic acids (K & K Laboratories, Inc., Plainview, N.Y.), were used as supplied because methyl esters prepared from these compounds showed no contamination as determined by GLC. Diethylene, triethylene, and tetraethylene glycols (Jefferson Chemical Company, Houston, Texas) were used as supplied because they contained no impurities detectable by GLC. Succinic acid (J. T. Baker Chemical Co., Phillipsburg, N.J.) and sodium acetate (Fisher Scientific Co., Pittsburgh, Pa.) were used as supplied. All other chemicals used were reagent grade.

Procedure. Experiments were conducted in oversize Warburg flasks (Aminco, 130-ml) containing 30 ml of the sterile basal salts medium. Replicate sets of four flasks containing a fourfold range of substrate concentrations, approximately 20, 40, 60, and 80 μmoles per 30 ml, were prepared with 0.5 ml of 50% KOH in the center wells to trap carbon dioxide. Inocula were placed in the side arms, bringing the total fluid volume to 31 ml and the total culture volume to 30.5 ml. After equilibration at 30 C, the cells were tipped in and oxygen uptake was followed manometrically as the bacteria multiplied until semilogarithmic plots of oxygen uptake versus time were no longer linear, as suggested by Elsden (*personal communication*).

Flasks were then removed from the apparatus, cell crops determined, and the media analyzed for residual substrate and possible end products of metabolism.

Determination of cell yields. Yields of cells grown on water-soluble substrates were determined by measurement of optical density at 420 mμ on a junior spectrophotometer (Coleman Instruments, Maywood, Ill.) and by reference to standard curves relating optical density and dry weight of cells. Because 1-dodecanol-grown cultures exhibited a spurious turbidity as a result of the insolubility of the substrate (17), yields in this system were determined directly. Cells were harvested by centrifugation at 37,000 × *g* for 30 min; they were washed twice by resuspension and centrifugation in sterile, distilled water (at which time gas chromatographic analysis showed no 1-dodecanol remaining in the supernatant fluid); and they were then weighed after drying to constant weight at 105 C.

In all cases, yields were corrected for weight of inoculum.

Preparation of standard curves. Standard curves were prepared for cells grown on each substrate. Adapted cells were harvested as described above, and were washed free of salts and substrates with sterile, distilled water. Triplicate, 30.5-ml suspensions were prepared with arbitrary optical densities over the range between 0 and 0.5 at 420 mμ. Each of these suspensions was transferred quantitatively to a tared 100-ml beaker and weighed after drying to constant weight at 105 C. Water blanks and tare controls were included in the weighing procedures. Best-fitting lines were calculated by the method of least squares.

Determination of residual substrate. The method of internal standardization, which is considered to be the most precise means of quantitation (2), was used to determine residual substrates. Each internal standard was chosen to be of the same functional type as the compound of interest and to elute near this compound. Our previous studies have shown this to minimize errors induced by sample treatment (19).

Media from experiments in which 1-dodecanol or an organic acid was used as substrate were extracted with hexane after acidification and addition of an appropriate internal standard. 1-Dodecanol was determined by direct injection of the hexane extract into the gas chromatograph, and the organic acids were determined after esterification of the dried hexane extract with methanol-perchloric acid (20).

Media in which the various ether glycols had been used as substrates were evaporated to near 1 ml after addition of the appropriate internal standard, and samples of the aqueous solutions were injected directly into the instrument.

Concentration of the remaining substrate was determined in all cases by calculation of the ratio of the peak area for the compound of interest to the peak area for the internal standard. For each substrate, a standard curve was established relating the peak area ratios to concentrations encompassing a range of 0.5 to 20 μmoles per 30 ml.

Typically, residual substrate was determined to be between 0 and 5% of the original concentration. In a few instances, residual substrate concentrations were determined to be 25 to 40% of the original concentration. In all cases, substrate utilized was considered to be the difference between original and residual concentrations.

No end products detectable by gas-liquid chromatography were found.

Gas-liquid chromatography. All analyses were performed by using a model 400 biomedical gas chromatograph (F & M Scientific Div., Hewlett-Packard Co., Avondale, Pa.) with a hydrogen flame ionization detector. Peak area measurements were made with a Disc Integrator (Disc Instruments, Santa Ana, Calif.) installed in the instrument.

1-Dodecanol was determined in the presence of undecanol on a 4 ft × ¼ inch outside diameter (1.22 m × 0.64 cm) copper column containing Chromosorb W coated with polyethylene glycol 1,000 (PEG-1,000), 20%, w/w (12). Oven temperature was 125 C; injec-

tion port, 298 C; and detector, 298 C. Helium flowrate was 40 ml/min.

Benzoic and phenylacetic acids served reciprocally as internal standards. Analyses of the methyl esters were performed with a 13 ft × ⅛ inch outside diameter (3.96 m × 0.32 cm) stainless-steel column containing Aeropak 30 coated with Tetracyanoethylated pentaerythritol (TCEPE), 3%, w/w (Varian Aerograph, Walnut Grove, Calif.). Oven temperature was 132 C; injection port, 275 C; and detector, 305 C. Carrier flow was 40 ml/min.

Phenylglyoxylic acid was determined with 2-(*o*-chlorophenoxy)-propionic acid as internal standard. The methyl esters were analyzed on a 6 ft × ¼ inch outside diameter (1.83 m × 0.64 cm) copper column containing Gas Chrom Q coated with PEG-1,000, 20%, w/w. Temperatures were: oven, 130 C; injection port, 268 C; and detector, 302 C. Helium flowrate was 35 ml/min.

Acetic acid was determined in the presence of propionic acid. Spent medium with added internal standard was reduced in volume to near 1 ml. The medium was acidified with 6 N HCl, and the aqueous solution was injected into the gas chromatograph. Separation was obtained with a 4 ft × ¼ inch outside diameter (1.22 m × 0.64 cm) glass column containing Porapak Q. Temperatures were: oven, 180 C; injection port, 340 C; and detector, 320 C; flowrate was 60 ml/min.

Glycerol served as internal standard for determinations of residual tetraethylene glycol, which were carried out on a 4 ft × ¼ inch outside diameter (1.22 m × 0.64 cm) glass column of Gas Chrom Q coated with PEG-1,000, 20%, w/w. Temperatures were: oven, 148 C; injection port, 320 C; and detector, 300 C; carrier flow was 40 ml/min.

Diethylene and triethylene glycols served reciprocally as internal standards, with the same conditions, except that the oven temperature was lowered to 120 C.

Calculations. On the basis of the data obtained, the following calculations were made for each individual sample: yield (grams dry weight of cells) per mole of substrate utilized (Y_{sub}), yield per gram atom of substrate carbon utilized (Y_{carb}), yield per mole of oxygen consumed (Y_{O_2}), moles of oxygen consumed per mole of substrate utilized (O_2/sub), and yield per equivalent of available electrons in the substrate utilized ($Y_{av\ e^-}$). The 95% confidence intervals were calculated by Student's *t* formula (7).

RESULTS

Variation of optical density versus dry-weight curves with respect to type of substrate. Figure 1 shows that the type of substrate has an effect on the light-scattering ability or the weight of cells, or both. Divergence of the standard curves would be expected between organisms, but not so significantly between classes of compounds used as substrates for the same organism.

Typical results. Table 1 shows the type and magnitude of results obtained from representative sets from the various test systems. The total number of determinations for each test system is indicated in Table 2.

Correlations between various parameters. Table 2 summarizes the correlations between the various parameters of these systems. Data in the column headed *n* represent the number of individual samples in the various test systems. Those in the column headed *e⁻/mole* represent the number of bonding electrons in a molecule of a particular compound which are not involved in a molecular orbital with oxygen in the structure of the compound. These, then, are defined as "available electrons," i.e., electrons available either for transfer to oxygen in respiration, or for incorporation and nonrespiratory reductions involved in synthesis.

Four equivalents of electrons are required to reduce 1 mole of oxygen; therefore, the number of available electrons in a compound may be conveniently calculated by multiplying by four the number of moles of oxygen required to balance the chemical equation of complete oxidation to carbon dioxide and water.

It can be seen from Table 2 that Y_{sub} varies widely among substrates, as might be expected; of the substrates representing the extremes, acetate (Y_{sub} = 23.5 g/mole) is a two-carbon com-

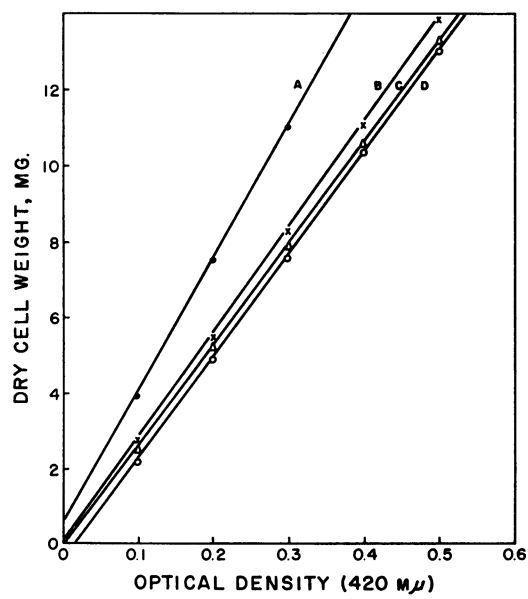

FIG. 1. *Typical regression lines of dry cell weight versus optical density for various substrates. (A) Isolant TEG-5 (●) grown on diethylene, triethylene, and tetraethylene glycols, Y (mg) = 35.5 × (optical density) + 0.41; (B) Pseudomonas C12B (×) grown on benzoic acid, Y = 27.9 × −0.08; (C) C12B (△) on succinate and acetate, Y = 27.0 × −0.21; (D) C12B (○) on phenylacetic acid, Y = 27.3 × −0.57.*

TABLE 1. *Typical data from representative sets of samples from selected test systems*

Substrate supplied (μmoles)	Substrate utilized (μmoles)	Oxygen consumed (μmoles)	Dry wt of cells, net (mg)	Y_{sub} (g/mole)	Y_{carb} (g per gram atom)	Y_{O_2} (g/mole)	O_2/sub (mole/mole)
Benzoic acid							
17.32	17.32	59.78	1.45	83.7	12.0	24.2	3.45
34.64	34.64	111.43	3.15	90.9	13.0	28.3	3.22
51.96	51.96	178.26	4.45	85.6	12.2	25.0	3.43
69.28	69.28	237.72	5.95	85.9	12.3	25.0	3.43
Phenylacetic acid							
18.53	18.40	63.84	2.30	125.0	15.6	36.0	3.47
37.06	37.06	116.61	4.35	117.4	14.7	37.3	3.15
55.59	55.59	184.96	6.20	111.5	13.9	33.5	3.33
74.12	60.94	192.72	6.20	101.7	12.7	32.2	3.16
Succinate plus acetate							
18.37	18.37	45.40	1.15	62.6	10.4	25.3	2.47
36.74	36.74	93.12	2.58	70.2	11.7	27.7	2.53
55.11	55.11	141.20	4.10	74.4	12.4	29.0	2.56
73.48	73.48	188.39	5.77	78.5	13.1	30.6	2.56
1-Dodecanol							
13.42	12.09	108.75	3.00	248.1	20.7	27.6	9.00
26.84	25.79	198.70	6.50	252.0	21.0	32.7	7.70
40.25	36.18	290.49	7.70	212.8	17.7	26.5	8.03
53.67	52.13	334.29	10.00	191.8	16.0	29.9	6.41
Tetraethylene glycol							
12.87	12.87	80.31	1.50	116.6	14.6	18.7	6.24
25.74	25.74	157.54	3.00	116.6	14.6	19.0	6.12
38.67	38.67	284.37	4.00	103.6	13.0	14.1	7.37
51.48	51.48	325.36	5.00	96.4	12.0	15.4	6.32

pound, whereas 1-dodecanol (Y_{sub} = 217 g/mole) is a 12-carbon compound.

Consideration, then, of the yields on the basis of gram atom of carbon utilized might be expected to provide a common basis for comparison. That this is not the case can be seen by the variation of Y_{carb}, ranging from 10.6 g per gram atom for succinate to 18.1 g per gram atom for dodecanol.

As Johnson (8) has pointed out, yield per mole of oxygen consumed might be expected to provide useful insights, because energy made available from aerobic processes is approximately proportional to quantity of oxygen consumed. That this proportionality is only approximate is indicated in the current study by the variation in Y_{O_2}, ranging from near 21 g per mole in some instances to 31 g per mole in others. Apparently, the normalizing factor in this case would be the efficiency with which the energy of oxidation is utilized by the cells, namely, the overall phosphorylation efficiency as expressed by P-O ratios. Such data are lacking at this time.

However, a consideration of what might be termed the average degree of reduction of the carbon in a compound, as expressed by the number of available electrons in the compound, provides a common basis for comparison of the various compounds. This consideration of "degree of reduction of carbon" has also been suggested by Gunsalus and Shuster (6).

It can be seen that the yield per equivalent of available electrons utilized (either by transfer to oxygen or by incorporation), $Y_{av\ e^-}$, is near 3 g per equivalent for the compounds tested, with a mean value of 3.14 g per equivalent.

This yield coefficient appears to have applicability and predictive value to systems other than ours. Table 3 presents values of cell yields found in the literature which are here compared to the value predicted on the basis of equivalents of available electrons. The conditions under which these reported yields were obtained were either not specified in the sources cited, or, when specified, were different from the conditions used in our studies.

DISCUSSION

The column listing the values of moles of oxygen consumed per mole of substrate utilized (O_2/sub) in Table 2 does not have a direct bearing on cell-yield considerations, but does provide interesting data in its own right. These values represent 40 to 50% of the theoretical oxygen required for complete oxidation of a mole of substrate to carbon dioxide and water for all

TABLE 2. *Summary of experimental values calculated for each sample*[a]

Substrate	n	Y_{sub}	Y_{carb}	Y_{O_2}	O_2/sub	e^-/mole	$Y_{av\ e^-}$[b]
Benzoate	24	86.8 ± 3.1[c]	12.4 ± 0.4	25.5 ± 1.1	3.46 ± 0.15	30	2.89 ± 0.10
Phenylglyoxylate	16	102.2 ± 8.1	12.8 ± 1.0	25.4 ± 1.3	4.06 ± 0.30	32	3.20 ± 0.25
Phenylacetate	24	111.0 ± 4.1	13.9 ± 0.5	29.9 ± 1.4	3.73 ± 0.14	36	3.08 ± 0.12
Succinate plus acetate	16	72.2 ± 3.5	12.1 ± 0.6	28.1 ± 1.3	2.54 ± 0.04	22	3.28 ± 0.16
Acetate	12	23.5 ± 1.7	11.8 ± 0.8	21.4 ± 1.6	1.11 ± 0.05	8	2.94 ± 0.21
Succinate	12	42.3 ± 2.8	10.6 ± 0.7	31.4 ± 2.8	1.40 ± 0.07	14	3.02 ± 0.31
1-Dodecanol	38	217.0 ± 39.6	18.1 ± 3.4	31.8 ± 3.9	7.41 ± 1.12	72	3.01 ± 0.56
Diethylene glycol	4	58.0 ± 2.3	14.5 ± 0.5	18.7 ± 1.8	3.10 ± 0.16	20	2.90 ± 0.13
Triethylene glycol	28	103.1 ± 5.8	17.2 ± 0.9	23.2 ± 0.9	4.40 ± 0.12	30	3.44 ± 0.17
Tetraethylene glycol	42	129.9 ± 6.6	16.2 ± 1.0	21.6 ± 1.6	6.01 ± 0.22	40	3.25 ± 0.16

[a] Abbreviations used: n = number of samples; Y_{sub} = grams of dry-cell yield per mole of substrate utilized; Y_{carb} = grams of dry-cell yield per gram atom of carbon utilized; Y_{O_2} = grams of dry-cell yield per mole of oxygen consumed; O_2/sub = moles of oxygen consumed per mole of substrate utilized; e^-/mole = equivalents of available electrons per mole of substrate; $Y_{av\ e^-}$ = grams of dry-cell yield per equivalent of available electrons.

[b] Calculated for 216 individual determinations, $Y_{av\ e^-}$ = 3.03 = m (3.14) = 3.25.

[c] Also can be expressed as: 83.7 = m = 89.9. These values are the limits of the 95% confidence interval as calculated by the Student's t test (7).

TABLE 3. *Comparison of molar growth yields of various microorganisms with molar growth yields predicted on the basis of available electrons*

Substrate	Organism	e^-/mole	Predicted Y_{sub}	Reported Y_{sub}	Source
Glucose	*Candida utilis*	24	75.4	91.9[a]	Hernandez[b]
	Pseudomonas fluorescens			66.6[a]	
	Pseudomonas C_1			78	S. F. Carson (*personal communication*)
	Pseudomonas C_5			79	
	Arthrobacter globiformis			94	Morris (14)
	Escherichia coli			90	
Acetate	*C. utilis*	8	25.1	21.6[a]	Hernandez[b]
	P. fluorescens			16.8[a]	
	Pseudomonas C_1			19	S. F. Carson (*personal communication*)
	Pseudomonas C_5			20	
Ethyl alcohol	*C. utilis*	12	37.7	31.3[a]	Hernandez[b]
Octadecane	*P. aeruginosa*	110	345.4	152.7[a]	Ertola et al. (4)
	Pseudomonas species			262.1[a]	Wodzinski[b]
Succinate	*Pseudomonas* C_1	14	44.0	38	S. F. Carson (*personal communication*)
	Pseudomonas C_5			34	

[a] Calculated from cell yield per gram of substrated utilized.

[b] From Johnson (8).

substrates except acetate and the ether glycols, for which O_2/sub represents 56 and 62% of the theoretical oxygen, respectively.

A pattern which becomes apparent on inspection is the difference of O_2/sub of 0.5 moles/mole between benzoate and phenylglyoxylate, which differ by two equivalents of available electrons. If *Pseudomonas* C12B might be presumed to utilize these compounds by the pathway outlined by Gunsalus, Stanier and Gunsalus, and Hayaishi and Stanier as cited by Knox (11), then phenylglyoxylate is decarboxylated to benzaldehyde, which is dehydrogenated to benzoic acid and subsequently degraded via catechol, *cis-cis*-muconic acid, and β-ketoadipic acid to succinate and acetyl coenzyme A. This difference of 0.5 mole of O_2 consumed per mole of substrate utilized could then be accounted for by the dehydrogenation of benzaldehyde to benzoic acid.

Phenylacetic acid appears to be metabolized by *Pseudomonas* C12B through another pathway, most likely via homogentisic acid to fumarate

and acetoacetate (11). Thus, the O_2/sub value for this compound does not appear to be related to those of benzoate and phenylglyoxylate.

A more striking pattern can be seen in the oxygen consumed per mole of substrate utilized for the ether glycols, in which the O_2/sub ratios of near 3, 4.5, and 6 for diethylene, triethylene, and tetraethylene glycols, respectively, reflect a multiplicity related to the number of two-carbon fragments in the molecule. By extrapolation, this reveals a requirement of approximately 1.5 moles of oxygen per ethylene glycol fragment.

A further consistency is found in the additivity of the O_2/sub ratios for succinate and acetate to agree with the value for the equimolar succinate-acetate system. This indicates that the two substrates are used simultaneously in a 1:1 ratio.

Although it has been stated that heat of combustion of a substrate has no meaningful relationship with metabolic processes, it is interesting to consider heats of combustion on the basis of the available electrons. Kharasch (9) and Kharasch and Sher (10) have made an extensive study of ΔH values and have concluded that the energy involved in the transfer of an electron from a methane-type bond to a bond with oxygen amounts to 26.05 kcal per equivalent. By using this value for the methane electron and by applying a series of correction factors for the kinds and numbers of bond configurations other than methane, these authors were able to calculate heats of combustion of an exceedingly large variety of compounds in almost exact agreement with reported experimental values.

Considering a total of 18 compounds, including the substrates we have used and a variety of additional compounds including fatty acids, amines, amino acids, peptides, carbohydrates, and hydrocarbons, we have calculated, from literature values of ΔH (9, 10), heats of combustion per available electron having a mean of 26.53 kcal per equivalent.

From the first law of thermodynamics, change in enthalpy is dependent only on initial and final states and is independent of pathway (3); therefore, one may consider that the substrate provides electrons with a potential energy of 26.53 kcal per equivalent. Those which are incorporated as cellular material may be considered to remain at approximately the same energy potential. Those which are transferred to oxygen provide energy, a portion of which is trapped by oxidative phosphorylation or by some other means of generating high-energy compounds. This energy is subsequently used in the endergonic reactions of the cells (i.e., synthesis, transport, and polymer synthesis). The balance is expended as heat.

Combining our observed yield value of 3.14 g (dry) weight of cells per equivalent of available electrons utilized with the calculated mean energy of an equivalent of available electrons, 26.53 kcal per equivalent, one can calculate another predictive factor likely to be useful in considerations of systems for which exact molecular weights and therefore molar concentrations are not known. This combined factor, 3.14 g per equivalent utilized divided by 26.53 kcal per equivalent, has the value and dimensions of $Y_{K_{cal}} = 0.118$ g of dry weight per Kcal utilized.

The applicability of this conversion factor is obvious in that it provides a means of testing the validity of this concept with respect to mixtures of substrates such as might be found in natural waters.

We may conclude, then, that the yield of bacterial cells obtained from growth on those organic compounds which are completely utilized aerobically for both energy and assimilation appears to be specifically a function of the available electrons in the substrate compound. The yield seems further to be independent of either the type of compound or of the microbial organism. A yield of 3 g of cells per equivalent of available electrons has been observed to be a constant in these studies. In view of the fact that yields predicted on the basis of this proposed constant are usually equal to or greater than the yield values reported in the literature, it would appear that a $Y_{av\ e^-}$ near 3 g per equivalent might represent a measure approaching the maximal yield obtainable from aerobic growth on limiting amounts of synthetic organic compounds used as sole sources of carbon and energy.

If additional investigation bears out this constancy or provides data for accepting a different constant, the predictive value will be great. Emphasis simply on degrading waste material will probably diminish in the future and be replaced by a desire to transform waste organic substances as well as hydrocarbons into protein and other nutritionally valuable substances. This concept has growing appeal. A constant which could be used to predict the probable yield from a given process would be useful for calculating costs of production, and thus feasibility.

It is necessary that this newly proposed constant be further tested, particularly under the conditions of continuous propagators.

Studies are currently under way to attempt to evaluate these data with regard to the energy relationships involved, and to correlate, if possible, these values for aerobic growth with the adenosine triphosphate yield calculated for anaerobes by Bauchop and Elsden (1).

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LITERATURE CITED

1. BAUCHOP, T., AND S. R. ELSDEN. 1960. The growth of micro-organisms in relation to their energy supply. J. Gen. Microbiol. **23**:457–469.
2. DAL NOGARE, S., AND R. S. JUVET, JR. 1962. Gas-liquid chromatography, theory and practice, p. 256. Interscience Publishers, New York.
3. DANIELS, F., AND R. A. ALBERTY. 1955. Physical chemistry. John Wiley and Sons, Inc., New York.
4. ERTOLA, R. J., M. D. LILLY, AND F. C. WEBB. 1965. Production of cell tissue from hydrocarbons by a microbiological process. Biotechnol. Bioeng. **7**:309–319.
5. FINCHER, E. L., AND W. J. PAYNE. 1962. Bacterial utilization of ether glycols. Appl. Microbiol. **10**:542–547.
6. GUNSALUS, I. C., AND C. W. SHUSTER. 1961. Energy-yielding metabolism in bacteria. *In* I. C. Gunsalus and R. Y. Stanier [ed.], The bacteria, vol. 2. Academic Press, Inc., New York.
7. HUNTSBERGER, D. V. 1961. Elements of statistical inference. Allyn and Bacon, Inc., Boston.
8. JOHNSON, M. J. 1967. Growth of microbial cells on hydrocarbons. Science **155**:1515–1519.
9. KHARASCH, M. S. 1929. Heats of combustion of organic compounds. Bur. Std. J. Res. **2**:359–430.
10. KHARASCH, M. S., AND B. SHER. 1925. The electronic conception of valence and heats of combustion of organic compounds. J. Phys. Chem. **29**:625–658.
11. KNOX, W. E. 1961. Degradation of aromatic compounds, p. 596–601. *In* C. Long [ed.], Biochemist's handbook. D. Van Nostrand Co., Inc., Princeton, N.J.
12. MAYBERRY, W. R., AND G. J. PROCHAZKA. 1965. Polyethylene glycol 600: an efficient liquid phase for alcohols and free fatty acids. J. Gas Chromatog. **3**:232–234.
13. MONOD, J. 1942. Recherches sur la croissance des cultures bacteriennes. Herman et Cie., Paris.
14. MORRIS, J. G. 1960. Studies of the metabolism of *Arthrobacter globiformis*. J. Gen. Microbiol. **22**:564–582.
15. PAYNE, W. J., AND V. E. FEISAL. 1963. Bacterial utilization of dodecyl sulfate and dodecyl benzene sulfonate. Appl. Microbiol. **11**:339–344.
16. PAYNE, W. J., G. J. PROCHAZKA, AND W. R. MAYBERRY. 1967. Measurement of yields from cultures growing on synthetic organic compounds. J. Gen. Microbiol. **45**:6–7.
17. PROCHAZKA, G. J., W. R. MAYBERRY, AND W. J. PAYNE. 1967. Model systems for studying energy yields from synthetic organic compounds. Develop. Ind. Microbiol. **8**:167–178.
18. STORCK, R., AND R. Y. STANIER. 1956. Energy-yielding metabolism in bacteria, p. 1–58. *In* I. C. Gunsalus and R. Y. Stanier [ed.], The bacteria, vol. 2. Academic Press, Inc., New York.
19. WILLIAMS, J. P., W. R. MAYBERRY, AND W. J. PAYNE. 1966. Metabolism of linear alcohols with various chain lengths by a *Pseudomonas* species. Appl. Microbiol. **14**:156–160.
20. WYNNE, R. B., J. A. SCHMIT, AND G. R. UMBREIT. 1965. Perchloric acid catalyzed reactions. I. Methylation of fatty acids. F & M Scientific Div., Hewlett-Packard Co., Avondale, Pa.