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The effect of cell metabolism on biomass yield during the growth on various substrates

I. G. Minkevich

G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms,
Russian Academy of Sciences,
Nauki Ave. 5, Pushchino, Moscow Region, 142290, Russia

E-mail: minkevich@ibpm.pushchino.ru

Bioenergetic regularities determining the maximal biomass yield in aerobic microbial growth on various substrates have been considered. The approach is based on the method of mass-energy balance and application of GenMetPath computer program package. An equation system describing the balances of quantities of 1) metabolite reductivity and 2) high-energy bonds formed and expended has been formulated. In order to formulate the system, the whole metabolism is subdivided into constructive and energetic partial metabolisms. The constructive metabolism is, in turn, subdivided into two parts: forward and standard. The latter subdivision is based on the choice of nodal metabolites. The forward constructive metabolism is substantially dependent on growth substrate: it converts the substrate into the standard set of nodal metabolites. The latter is, then, converted into biomass macromolecules by the standard constructive metabolism which is the same on various substrates. Variations of flows via nodal metabolites are shown to exert minor effects on the standard constructive metabolism. As a separate case, the growth on substrates requiring the participation of oxygenases and/or oxidase is considered. The bioenergetic characteristics of the standard constructive metabolism are found from a large amount of data for the growth of various organisms on glucose. The described approach can be used for prediction of biomass growth yield on substrates with known reactions of their primary metabolization. As an example, the growth of a yeast culture on ethanol has been considered. The value of maximal growth yield predicted by the method described here showed very good consistency with the value found experimentally.

Keywords: biomass growth yield, cell metabolism, constructive metabolism, energetic metabolism, nodal metabolites, mass-energy balance

АНАЛИЗ И МОДЕЛИРОВАНИЕ СЛОЖНЫХ ЖИВЫХ СИСТЕМ

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Влияние метаболизма клеток на выход биомассы при росте на различных субстратах

И. Г. Минкевич

ФГБУН Институт биохимии и физиологии микроорганизмов им. Г. К. Скрябина
Российской академии наук,
Россия, 142290, Московская область, г. Пущино, пр-кт Науки, д. 5

E-mail: minkevich@ibpm.pushchino.ru

Рассмотрены биоэнергетические закономерности, определяющие максимальный выход биомассы при аэробном росте микроорганизмов на различных субстратах. Подход основан на методе материально-энергетического баланса и использовании пакета компьютерных программ *GenMetPath*. Сформулирована система уравнений, описывающих балансы количеств (1) восстановленности метаболитов и (2) образованных и затраченных макроэргических связей. Чтобы сформулировать эту систему, целостный метаболизм разделен на конструктивный и энергетический парциальные обмены. Конструктивный обмен, в свою очередь, разделен на две части: передний и стандартный конструктивные обмены. Последнее разделение основано на выборе узловых метаболитов. Передний конструктивный обмен существенно зависит от субстрата роста: он превращает субстрат в стандартный набор узловых метаболитов. Последний затем превращается в макромолекулы биомассы стандартным конструктивным обменом, который одинаков на различных субстратах. Показано, что вариации потоков через узловые метаболиты оказывают незначительное влияние на стандартный конструктивный обмен. В качестве отдельного случая рассмотрен рост на субстратах, требующих участия оксигеназ и/или оксидаз. Биоэнергетические характеристики стандартного конструктивного обмена найдены из большого числа данных для роста различных организмов на глюкозе. Описанный подход может быть использован для предсказания выхода биомассы на субстратах с известными реакциями их первичной метаболизации. В качестве примера рассмотрен рост культуры дрожжей на этаноле. Значение максимального выхода, предсказанное описанным здесь методом, показало хорошее соответствие значению, найденному экспериментально.

Ключевые слова: выход биомассы, метаболизм клеток, конструктивный обмен, энергетический обмен, узловые метаболиты, материально-энергетический баланс

Introduction

Metabolism of living cells is an example of a highly complex natural system. Its comprehension as a whole needs some integral, gross characteristics. The yield of cell biomass from a substrate — a source of matter and energy — is one of such indices widely used in microbiology and biotechnology. Usually it is expressed as a mass yield, viz., the ratio of the dry mass of cells grown to the mass of substrate consumed. Earlier we had introduced another index of cell growth performance, the energetic yield [Minkevich, Eroshin, 1973; Erickson et al., 1978; Minkevich, 1985]. It estimates the efficiency of conversion of substrate energy store into the energy incorporated into biomass. Both indices are interconnected by a simple proportionality, the coefficient of which depends on the elemental composition of substrate and dry cell biomass (see below). Since the energy is closely connected with the driving force of biochemical processes, the energetic yield is the basic measure of growth efficiency from which the values of other indices can be obtained.

An important task in this field is to compare the efficiency of matter and energy conversion during the growth of microbial cultures on different substrates. A related problem is theoretical prediction of biomass yield from a given substrate. The latter problem is essential in both basic and applied aspects. Its solution makes it possible to elucidate the general regularities of cell bioenergetics. It is topical in the design of biotechnological processes, e.g., in choosing the raw material for biomass production or estimating the amount of cell biomass which can be expected to be grown from a given substrate.

Previous works in this field showed an essential divergence between theoretical estimates of cell yield and its experimental values (for review see [Stouthamer, 1979]). Those estimates were based on calculations of the amount of high-energy bonds necessary for biosynthesis of all known cellular components. We proposed another approach, prediction of biomass yield from a given substrate using the data obtained with another, well-studied substrate [Minkevich, 1985; Minkevich et al., 2013], using differences in the stoichiometry of metabolization of both substrates. The present work is a further development of this approach.

Basic notions and quantities

1°. *Interrelation between balances of mass and reductivity* [Minkevich, 1982, 2005; Minkevich et al., 2013]. Estimation of the amount of a substance in terms of a quantity evaluating its reductivity gives advantages in formulating and solving a number of problems of the whole metabolism balance during the cell growth. Earlier we had introduced a generalized measure of substance reductivity, redoxon (RO), based on the reference zero level of reductivity common for all chemical compounds. The zero level of reductivity is assigned to water, carbon dioxide, ammonia, etc. Accordingly, *the biochemical zero level* of energy, enthalpy and free energy is also assigned to these compounds. Three properties of the redoxon are important for application of this notion.

A. The energy store of organic compounds relative to the mentioned zero level is proportional to the content of redoxons, the proportionality coefficient being close to a constant, approx. 112 kJ/eq. RO. Deviations of this coefficient for various organic compounds are rather small. This regularity is also related to enthalpy and standard Gibbs free energy.

B. The amount of any substance, except those having a zero reductivity level (i.e., not possessing redoxons), expressed in moles or grams, is proportional to the number of its redoxons, i.e., to the amount of its reductivity. The number of redoxons in 1 mole of a compound of elemental composition $C_{n_C} H_{n_H} O_{n_O} N_{n_N}$ is $\alpha^{RO} = 4n_C + n_H - 2n_O - 3n_N$ (molar reductivity). The number of redoxons in an arbitrary number of moles, n^{mol}, equals $N^{RO} = \alpha^{RO} n^{mol}$. It is also suitable to introduce the mass reductivity of organic substances by expressing their elemental composition per 1 C-mol (mole of carbon atoms): $CH_{b_H} O_{b_O} N_{b_N}$. The number of redoxons in any organic substance (a single compound or a mixture, like, e.g., dry biomass) containing 1 mole of carbon, called the reductance degree of a sub-

stance, is $\gamma = 4 + b_H - 2b_O - 3b_N$. The redoxon number N^{RO} in a carbonaceous substance containing N^{carbon} atoms C equals $N^{RO} = \gamma N^{carbon}$. Denoting the carbon mass fraction in a substance as σ, we have that m grams of the substance contain $N^{RO} = \dfrac{\sigma \gamma}{12} m$. The mass dry cell yield from a substrate S is $Y_{X/S} = \dfrac{m_B}{m_S}$, where m_S and m_B are the masses of substrate consumed and dry biomass grown. From the mentioned here, the biomass redoxon yield from the substrate, $\eta_{X/S}$, equals $\eta_{X/S} = \dfrac{\sigma_B \gamma_B}{\sigma_S \gamma_S} Y_{X/S}$.

It was found that the energy level of redoxons in dry biomass is quite the same as that in individual organic compounds [Minkevich, Eroshin, 1973; Erickson et al., 1978; Minkevich, 1982, 2005]. Therefore, $\eta_{X/S}$ can be interpreted as the biomass energy yield. Our special investigation as well as data by other researchers (for review see [Minkevich, 2005]) have shown that various microbial species (yeasts, bacteria, etc.) possess the same value of $\sigma_B \gamma_B = 1.9$ with small variations. The reductance degree of biomass carbon is also close to a constant value $\gamma_B = 4.2$. Exceptions are oleaginous microorganisms, i.e., those that produce and accumulate large amounts of lipids inside cells (a rare group of microorganisms) [Minkevich et al., 2010] and resinous components of some kinds of wood [Patel, Erickson, 1981].

C. Redoxons fit a specific conservation law: for any individual reaction or any reaction aggregate the overall number of RO's in the substrates equals the overall number of RO's in the products. For reactions where free oxygen is involved it should be taken into account that one O atom possesses –2 (minus two) redoxons. Therefore, direct participation of free oxygen in any reaction results in the loss of the corresponding RO number of <u>organic</u> reactants.

These properties of the redoxons make possible the consideration of the toichiometry of both single reactions and their conglomerates including the whole metabolism in terms of redoxon amounts. Recalculation of moles or grams to RO numbers can be easily made. Such analysis combines balances of matter and energy and, thus, describes the mass-energy balance.

2°. *Subdivision of the whole metabolism into partial metabolisms* [Minkevich, Eroshin, 1976; Minkevich, 1985. 2005]. This subdivision is necessary for analysis of the effect of cell energetics on the overall efficiency of substrates' conversion to biomass, i.e., for consideration of the biochemical background of the biomass yield.

Here we concentrate our investigation on the completely aerobic heterotrophic growth when cells obtain energy necessary for growth by means of substrate oxidation, the terminal oxidizer being free oxygen. A property of such a growth is the absence or a negligibly small quantity of organic products of metabolism. It is a widespread situation in nature.

The metabolism of growing cells forms *de novo* cell material which requires supply of energy. The supply is made in the form of high-energy bonds present in ATP, GTP and other energy carriers. They are necessary as a free energy source for macromolecule synthesis as well as for the operation of part of reactions in low-molecular metabolism. Besides, cells are subjected to degradation of their structures and to decay of cross-membrane gradients. Therefore, cells need to maintain their vital state by restoration processes which also require energy supply. Degradation and restoration form a specific kind of internal turnover of matter within cells which is called cell maintenance.

In accordance with the above said, the whole metabolism can be subdivided into the following partial metabolisms: constructive metabolism (from substrate to biomass), energetic metabolism forming high-energy compounds, and cell maintenance. The role of energetic metabolism during aerobic growth is carried out by cellular respiration: operation of the electron transport chain (ETC) and associated reactions. Subdivision of the metabolism into constructive and energetic parts has a parallel mode: flows via some reactions should be represented as sums of fractions ascribed to different partial metabolisms. Other reactions can be entirely included into one of the partial metabolisms. For exam-

ple, reactions of the glycolysis pathway belong to both constructive and energetic metabolism, and flows through them should be subdivided into corresponding parts.

Glucose is a substrate which is the most suitable for subdivision of its metabolism into constructive and energetic subsets (see below). For constructive metabolism we select reactions and parts of flows through them so as the amount of redoxons of the substrate consumed to be equal to that of biomass formed. Then, matter proceeds via the constructive metabolism from substrate to biomass on approximately the same energy level. The remaining metabolism acts as the energetic one. In this section we demonstrate how this separation produces a biochemical substantiation of the biomass yield.

Here and below we designate all metabolic flows, measured in redoxon units, by letter f with necessary superscripts or subscripts. All flows should be uniformly related to the existing dry cell substance (in this case the flows are the so called specific rates), or to the unit volume of cultivation medium with cells, or to anything else.

We use the following quantities describing input and output flows of the whole metabolism: $f^{(B)}$ for dry cell substance formation, $f^{(S)}$ for substrate consumption, and $f^{(ETC)}$ for oxygen consumption by the electron transport chain of cells. According to the law of RO conservation,

$$f^{(S)} = f^{(B)} + f^{(ETC)}. \tag{1}$$

Partial metabolisms interact by means of high-energy bond (HEB) balance. Let $f_{HEB}^{(E)}$ be the rate of high-energy bond <u>production</u> by the energetic metabolism. Interrelation of HEB and RO balances is described by the following gross stoichiometric coefficients: $\psi^{(B)}$, the number of HEB necessary for transfering 1 RO of substrate to biomass through the constructive metabolism (reactions of resynthesis participating in cell maintenance are not included here); $\psi^{(E)}$, the number of HEB formed as a result of 1 RO transfer through the energetic metabolism. Another quantity important for HEB balance is $f_{HEB}^{(maint)}$, the rate of HEB expenditure in all turnover processes constituting the cell maintenance. The HEB balance is described by the following expressions:

$$f_{HEB}^{(E)} = \psi^{(B)} f^{(B)} + f_{HEB}^{(maint)}, \tag{2}$$

where

$$f_{HEB}^{(E)} = \psi^{(E)} f^{(ETC)}. \tag{3}$$

The right-hand side of (2) expresses high-energy bond expenditures.

It should be taken into account that there are data [Neijssel, Tempest, 1976] indicating that the flow $f_{HEB}^{(maint)}$ consists of two summands one of which is proportional to the growth rate $f^{(B)}$:

$$f_{HEB}^{(maint)} = f_{HEB}^{(maint0)} + k_{HEB}^{(maint)} f^{(B)}. \tag{4}$$

In terms of the above rates, the biomass redoxon yield from the substrate is $\eta_{X/S} = \dfrac{f^{(B)}}{f^{(S)}}$. Solving Eqs. (1)–(4) gives the following expressions:

$$\frac{1}{\eta_{X/S}} = \frac{1}{\eta_{X/S}^{m}} + \frac{f_{RO}^{(maint)}}{f^{(B)}}, \tag{5}$$

where

$$\eta_{X/S}^{m} = \frac{\psi^{(E)}}{\psi^{(E)} + \tilde{\psi}^{(B)}}, \qquad \tilde{\psi}^{(B)} = \psi^{(B)} + k_{HEB}^{(maint)}, \qquad f_{RO}^{(maint0)} = \frac{f_{HEB}^{(maint0)}}{\psi^{(E)}}. \tag{6}$$

The quantity $f_{RO}^{(maint0)}$ is the $f^{(B)}$-independent part of the specific rate of substrate expenditure for cell maintenance. It is measured in redoxons transferred through the energetic metabolism for HEB supply necessary for this part of the maintenance processes. The quantity $\eta_{X/S}^{m}$ is the so called maximal growth yield to which the actual yield tends when $f^{(B)}$ is high compared with $f_{RO}^{(maint0)}$.

It is $\eta_{X/S}^{m}$ that is the most valuable as an estimate of biomass yield achievable during the cell population growth on a considered substrate.

3°. *Stoichiometry of sets of biochemical reactions*. It is described by a set of stoichiometric equations:

$$\sum_{r=1}^{R} \nu_{kr} z_r = b_k \quad \text{for } k = 1,...,K, \tag{7}$$

where R and K are, respectively, the total numbers of reactions and metabolites participating in the system of reactions, r and k are the sequence numbers of a given reaction and a given metabolite, the matrix ν_{kr} consists of reaction stoichiometric coefficients, every z_r is a flow via the r-th reaction, and every b_k is the rate of exchange by a k-th metabolite between the biochemical system and its surroundings. In addition to Eqs. (7), there exist a number of inequalities describing the thermodynamically permitted directions of irreversible reactions:

$$z_r^{irr} \geq 0. \tag{8}$$

Equations (7) with restrictions (8) make it possible to theoretically find metabolic pathways converting a preset substrate into given products and calculate high-energy bond balances of the pathways. Approaches providing transformation of the above interrelations and application of them for finding the pathway are described in detail in [Minkevich, 2015, 2016]. A computer program package based on the set of these approaches has been developed. Conditionally it can be called a generator of metabolic pathways (GenMetPath); this term is used below.

Statement of the problem

Requirements of cell matter biosynthesis in high-energy bonds can be calculated exactly using the GenMetPath package and the exact data on cell macromolecule composition. However, there are essential difficulties in the realization of this approach.

First of all, the composition of cell biomass varies for different strains.

Further, application of GenMetPath requires a local database containing stoichiometric equations of a sufficiently wide set of biochemical reactions (including the directions of all irreversible ones) from which this program package can choose the reactions and calculate flows through them. In the case of the constructive metabolism, the database should include all biochemical reactions providing the synthesis of all macromolecule subunits and their polymerization. This method of $\psi^{(B)}$ calculation requires much work necessary for compilation of the database. It is, in principle, possible.

There is another problem, the theoretical solution of which does not seem possible at present. According to Eqs. (6), calculation of $\eta_{X/S}^{m}$ requires a value of $\tilde{\psi}^{(B)} = \psi^{(B)} + k_{HEB}^{(maint)}$ which contains the summand $k_{HEB}^{(maint)}$. The latter joins the characteristics of many partial turnover processes the rates of which are unknown.

Therefore, more realistic is another approach for estimation of an achievable $\eta_{X/S}^{m}$ value for? a substrate of interest. We shall proceed from the fact that the growth on glucose is the best examined process of microbial growth. There are a lot of data concerning a great variety of microorganisms

(yeasts, bacteria, etc.) which, being grown aerobically on glucose under optimal conditions, display the same maximal growth yield: $Y_{X/S}^m = 0.5$ [Minkevich, 1985]. An exception are oleaginous microorganisms, i.e., those which produce and accumulate large amounts of intracellular lipids. Recalculation to redoxons gives: $\eta_{X/S}^m = 0.6$.

Efficiency of operation of the aerobic energy supplying system — the electron transport chain (ETC) plus ATP synthase — is described by a well-studied parameter P/O (ATP formed per one atom of oxygen consumed, i.e. per two redoxons). The ETC provides the major part of HEB's produced by the energetic metabolism. The P/O value under optimal conditions is usually $P/O \approx 3$ from which $\psi^{(E)} \approx 1.5$. Using the mentioned values of $\eta_{X/S}^m$ and $\psi^{(E)}$ we can find $\tilde{\psi}^{(B)}$ from the first Eq. (6).

It is known that the macromolecular composition of cells displaying $Y_{X/S}^m = 0.5$ as well as the amino acid composition of their protein, the base composition of their nucleic acids, etc., are rather variable but the latter has a minor effect on $Y_{X/S}^m$ and $\sigma_B \gamma_B$. Therefore, $\tilde{\psi}^{(B)}$ appears to be a metabolic parameter which is approximately constant for various cell species grown on glucose under optimal conditions. On the other hand, the metabolism of cells growing on different substrates differs in its initial part, viz., from the substrate to some metabolites — precursors of amino acids, monosaccharides, nucleotides and lipids, common for every substrate. Below we call them *nodal* metabolites. The mentioned part is a small portion of the whole metabolism.

The above stated allows bringing forward an assumption that $\tilde{\psi}^{(B)}$ consists of two summands. The first of them depends on the substrate. Another, from nodal metabolites to constituents of biomass, has the same value, at least, for many substrates and cell species. It should be emphasized that these properties of the second summand are related to optimal growth conditions. Conditions far from optimal may increase the value of $k_{HEB}^{(maint)}$ and, thus, may decrease $\eta_{X/S}^m$.

Then, we use the following principles as a background for predicting the value of cell biomass yield.

1. We subdivide the whole constructive metabolism into two parts: forward and standard. Then, the stoichiometric part of $\tilde{\psi}^{(B)}$, viz., $\psi^{(B)}$, becomes itself a sum of two parts:

$$\psi^{(B)} = \psi^{(B0)} + \psi^{(B1)}. \tag{9}$$

Here $\psi^{(B0)}$ and $\psi^{(B1)}$ are amounts of high-energy bonds _expended_ for the transfer of one eq. of RO through the standard and forward constructive metabolisms, respectively. According to the above said, $\psi^{(B0)}$ is practically constant whereas $\psi^{(B1)}$ varies depending on the growth substrate. The quantity $\psi^{(B)}$ is related to the total requirements of HEB's for biomass formation, and, as such, is always positive. The same applies to $\psi^{(B0)}$ since it is the major part of $\psi^{(B)}$ (see below). At the same time, $\psi^{(B1)}$ may be both positive when the forward metabolism requires high-energy bonds, and negative when this metabolism forms HEB's (e.g., in the case of glucose as a growth substrate; see below).

2. We consider $k_{HEB}^{(maint)}$, the rate coefficient of $f^{(B)}$-dependent HEB expenditure for cell maintenance, as also a constant (at optimal conditions) independent of organism species at least for aerobic growth. Then

$$\tilde{\psi}^{(B)} = \tilde{\psi}^{(B0)} + \psi^{(B1)}, \tag{10}$$

where

$$\tilde{\psi}^{(B0)} = \psi^{(B0)} + k_{HEB}^{(maint)} \tag{11}$$

and the summand $\psi^{(B1)}$ is quite the same as that in (9).

Thus, estimation of realistic value of $\eta_{X/S}^{m}$ (and, consequently, $Y_{X/S}^{m}$) consists of the following steps.

A. Finding the value of $\tilde{\psi}^{(B0)} = \psi^{(B0)} + k_{HEB}^{(maint)}$ using the value of cell energetic yield during the growth on the reference substrate, glucose: $\eta_{X/S}^{m} = 0.6$.

B. Calculation of $\psi^{(B1)}$ and, then, $\eta_{X/S}^{m}$ for a substrate of interest.

Effect of metabolic flow branching on the coupling of constructive metabolism and its energy supply

In this section we consider all substrates except those the metabolization of which is carried out by oxygenases or/and oxidases of the substrate or its derivatives. The latter substrates are considered in a separate section below.

Subdivision of the whole metabolism is to be made for writing the balance of high-energy bond (HEB) production and expenditure. It will serve as a basis for finding an expression of biomass yield via the bioenergetic characteristics of cell metabolism. Separate rates of the total HEB production are proportional to different redoxon flows. On the other hand, calculation of biomass yield requires the presence of only three flows in the whole HEB balance, viz., the overall substrate consumption — $f^{(S)}$, biomass formation — $f^{(B)}$, and the rate of HEB expenditures for cell maintenance. Other redoxon flows which describe any partial metabolic processes should be excluded from the overall equation of HEB balance. It will be made on the basis of redoxon balance equations. Derivation of RO and HEB balance equations is the subject of the present section.

1°. *Constructive metabolism*. Since the cell metabolism is really a cross-linked network, selection of nodal metabolites is not quite a simple task with a single solution. Nevertheless, it is possible to set a list of metabolites which can be considered as substrates for the standard constructive metabolism.

Based on the known biochemical data [BRENDA, ExPASy, KEGG], we choose the following nodal compounds.

Glucose is a substrate for synthesis of all monosaccharides necessary for production of other cellular sugars and polysaccharides. Glucose can be supplied directly when it is a growth substrate or is formed metabolically when cells utilize another substrate.

Acetyl-CoA is a source for lipid synthesis.

Amino acids are formed via several metabolites, 2-oxoglutarate, erythrose-4-phosphate, oxaloacetate, ribose-5-phosphate, 3-phosphoglycerate, phosphoenolpyruvate, and pyruvate.

Synthesis of nucleotides utilizes amino acids and monosaccharides, i.e., it succeeds the above mentioned processes.

These compounds (glucose, acetyl-CoA, and the mentioned precursors of amino acids) are accepted here as the nodal metabolites.

Consideration of the reactions making up a pathway from a growth substrate to a nodal metabolite shows that the latter is not a single product of such a pathway (see, e.g., Table 1). These transformations are often coupled with reduction of reductivity carriers (NAD^+ to NADH, $NADP^+$ to NADPH, ubiquinone Q to ubiquinol QH_2) which accept part of substrate redoxons. It takes place due to the fact that many growth substrates are more reduced than most nodal metabolites. On the other hand, there are growth substrates the reductivity of which is lower than that of the nodal metabolites. These substrates, on the contrary, require additional reductivity for such a conversion. Therefore, the balance of redoxon carriers should be taken into account in constructive metabolism subdivision into forward and standard parts.

We recall that all substance flows discussed here are measured in equivalents of metabolite redoxons per time unit (and per redoxons of existing dry cell substance or unit volume of cultivation

medium with cells). It is suitable, particularly, as the universal measure both for organic intermediates and for reductivity *transferred* by specific carriers (NAD, NADP, etc.).

1A. *Redoxon flows in the constructive metabolism*. Let nodal metabolites and the corresponding pathways leading from the growth substrate to these metabolites be numbered by p. The mentioned pathways corresponding to different p's usually contain many common reactions, especially those responsible for primary metabolization of the substrate. Full flows through these reactions are stoichiometrically subdivided into parts (summands) referred to different paths. Every p-th pathway accepts substrate flow $f_p^{(\text{SCons})}$ and forms on its output two RO flows: $f_p^{(\text{M})}$ related to the p-th nodal metabolite, and $f_p^{(\text{C1})}$ related to the reduced state of the reductivity carriers:

$$f_p^{(\text{SCons})} = f_p^{(\text{M})} + f_p^{(\text{C1})}. \tag{12}$$

Flows $f_p^{(\text{C1})}$ and $f_p^{(\text{M})}$ are coupled to each other by the following coefficient:

$$k_p^{(\text{C1})} = \frac{f_p^{(\text{C1})}}{f_p^{(\text{M})}}. \tag{13}$$

Every $k_p^{(\text{C1})}$ is a single-valued ratio determined by the stoichiometry of the *p*-th pathway.

The total substrate inflow to constructive metabolism, $f^{(\text{SCons})}$, equals $f^{(\text{SCons})} = \sum_p f_p^{(\text{SCons})}$, for which:

$$f^{(\text{SCons})} = f^{(\text{M})} + f^{(\text{C1})}, \tag{14}$$

where
$$f^{(\text{M})} = \sum_p f_p^{(\text{M})}, \quad f^{(\text{C1})} = \sum_p f_p^{(\text{C1})}. \tag{15}$$

The sum of all flows $f_p^{(\text{M})}$ plus the overall flow of redoxons on RO carriers, $f^{(\text{C0})}$, gives the total rate of biomass formation by the constructive metabolism, $f^{(\text{B})}$:

$$f^{(\text{B})} = f^{(\text{M})} + f^{(\text{C0})}. \tag{16}$$

Equations (14) and (16) describe the balances of the forward and standard constructive metabolism, respectively. They give the overall balance of the whole constructive metabolism:

$$f^{(\text{SCons})} = f^{(\text{B})} + f^{(\text{C1})} - f^{(\text{C0})}. \tag{17}$$

All the quantities participating in (17) are expressible via nodal metabolite flows $f_p^{(\text{M})}$. Using (13), we have:

$$f^{(\text{C1})} = \sum_p f_p^{(\text{C1})} = \sum_p k_p^{(\text{C1})} f_p^{(\text{M})}. \tag{18}$$

From (14) and (18):

$$f^{(\text{SCons})} = \sum_p \left(1 + k_p^{(\text{C1})}\right) f_p^{(\text{M})}. \tag{19}$$

An important role in the constructive metabolism belongs to interrelations between the quantities $f_p^{(\text{M})}$. To describe them, we introduce the distribution coefficients

$$\theta_p = \frac{f_p^{(\text{M})}}{f^{(\text{B})}}. \tag{20}$$

Substitution of (20) into (19) results in the following interrelation:

$$f^{(\text{SCons})} = f^{(\text{B})} \sum_p \left(1 + k_p^{(\text{C1})}\right)\theta_p. \tag{21}$$

Using (20) and the first interrelation (15), we transform Eq. (16) as follows:

$$f^{(\text{C0})} = f^{(\text{B})}\left(1 - \sum_p \theta_p\right). \tag{22}$$

Interrelations (18) and (20) yield the following:

$$f^{(\text{C1})} == f^{(\text{B})} \sum_p k_p^{(\text{C1})}\theta_p. \tag{23}$$

Equations (21)–(23) give expressions of the flows, which characterize the constructive metabolism as a whole, via the output flow $f^{(\text{B})}$.

Quantities θ_p may vary depending on the differences in biomass composition and pathways of macromolecule component synthesis. Effect of these variations is discussed below. Since the metabolism of glucose is chosen here as a reference point for quantities which are related to other substrates, we take for other substrates the same flow distribution via nodal metabolites as that for glucose.

1B. *High-energy bond flows in the constructive metabolism*. Let $f_{\text{HEB}}^{(\text{Cons})}$ be the flow of high-energy bonds necessary for operation of the whole constructive metabolism. It is expressed by the following equation:

$$f_{\text{HEB}}^{(\text{Cons})} = f^{(\text{B})}\psi^{(\text{B0})} + \sum_p f_p^{(\text{M})}\psi_p^{(\text{B1})}, \tag{24}$$

where $\psi^{(\text{B0})}$ is an overall stoichiometric coefficient describing HEB requirements of the standard constructive metabolism and $\psi_p^{(\text{B1})}$ are HEB/RO coefficients for HEB balance in the p-th pathway of the forward constructive metabolism (high-energy bonds per 1 RO of the p-th nodal metabolite).

A coefficient $\psi_p^{(\text{B1})} > 0$ when the p-th pathway requires high-energy bond supply and $\psi_p^{(\text{B1})} < 0$ when it produces HEB's.

A HEB/RO coefficient describing the overall HEB requirements for the functioning of the whole constructive metabolism, $\psi^{(\text{B})}$, is

$$\psi^{(\text{B})} = \frac{f_{\text{HEB}}^{(\text{Cons})}}{f^{(\text{B})}}. \tag{25}$$

Division of (24) by $f^{(\text{B})}$ and utilization of (20) yields the following expression for ψ_{B}:

$$\psi^{(\text{B})} = \psi^{(\text{B0})} + \sum_p \theta_p \psi_p^{(\text{B1})}. \tag{26}$$

Comparison of (26) with (9) shows that

$$\psi^{(\text{B1})} = \sum_p \theta_p \psi_p^{(\text{B1})}. \tag{27}$$

Adding $k_{\text{HEB}}^{(\text{maint})}$ to both parts of (26), we obtain:

$$\tilde{\psi}^{(\text{B})} = \tilde{\psi}^{(\text{B0})} + \sum_p \theta_p \psi_p^{(\text{B1})}. \tag{28}$$

2°. *Energetic metabolism.* It also consists of two parts: 1) the forward part from the substrate to reduced carriers of reductivity (NADH, NADPH, QH_2) plus inorganic compounds. CO_2, NH_3, etc., the latter do not carry available energy; 2) the electron transport chain (ETC) and ATP synthase; this system accepts reductivity from the mentioned carriers and produces ATP.

In the case of glucose, we determine the subdivision of the whole metabolism into its constructive and energetic parts so that $f^{(SCons)} = f^{(B)}$. It means that there is no interchange by redoxons between the constructive and energetic metabolisms. Then, according to (17), $\sum_p f_p^{(C1)} = f^{(C0)}$.

On other substrates this interchange is generally present, and $f^{(SCons)} \neq f^{(B)}$, $f^{(C1)} \neq f^{(C0)}$. It originates from the fact that reductivities of many substrates differ from that of glucose. In the case of a more reduced substrate (e.g., ethanol) the constructive metabolism produces excess redoxons carried by NADH etc. and these RO's proceed to ETC in addition to those arriving from the forward energetic metabolism. Oppositely, when the substrate is more oxidized than glucose (e.g., some Krebs cycle metabolites may be used as growth substrates), the forward energetic metabolism should supply part of NADH and NADPH into the constructive metabolism to compensate for the lack of reductivity.

When considering the growth on a substrate other than glucose, it should be taken into account that the efficiency of ATP production by the system ETC + ATP synthase on a given substrate may differ from that on glucose.

Let $f^{(SEn)}$ and $f^{(ETC)}$ be, respectively, redoxon flows via the energetic metabolism from the substrate to reduced carriers and from the latter to oxygen through the electron transport chain. Redoxon balance of aerobic growth on any substrate (without participation of oxygenases/oxidases) is described by the following equations:

$$f^{(ETC)} = f^{(SEn)} + f^{(C1)} - f^{(C0)}, \tag{29}$$

where $f^{(C1)} - f^{(C0)}$ is an RO flow from the interchange of the constructive metabolism with the energetic metabolism. Let us denote the ratios HEB/RO for both parts of the energetic metabolism as follows: $\psi^{(E1)}$ for the forward energetic metabolism and $\psi^{(ETC)}$ for the system ETC + ATP synthase. Then the rate of high-energy bonds produced by the whole energetic metabolism, $f_{HEB}^{(En)}$, equals

$$f_{HEB}^{(En)} = \psi^{(E1)} f^{(SEn)} + \psi^{(ETC)} f^{(ETC)}. \tag{30}$$

3°. *Whole metabolism.* The whole rate of substrate consumption, $f^{(S)}$, equals

$$f^{(S)} = f^{(SCons)} + f^{(SEn)}. \tag{31}$$

Full redoxon balance is as follows:

$$f^{(S)} = f^{(B)} + f^{(ETC)}. \tag{32}$$

Then the rate of HEB production by the energetic metabolism, as it follows from (30)–(32), equals

$$f_{HEB}^{(En)} = \left(\psi^{(E1)} + \psi^{(ETC)} \right) f^{(S)} - \psi^{(E1)} f^{(SCons)} - \psi^{(ETC)} f^{(B)}.$$

Excluding $f^{(SCons)}$ by the use of (21) results in

$$f_{HEB}^{(En)} = \left(\psi^{(E1)} + \psi^{(ETC)} \right) f^{(S)} - \left[\psi^{(E1)} \sum_p \left(1 + k_p^{(C1)} \right) \theta_p + \psi^{(ETC)} \right] f^{(B)}. \tag{33}$$

The overall balance of high-energy bonds is as follows:

$$f_{HEB}^{(En)} = f_{HEB}^{(Cons)} + f_{HEB}^{(maint)}. \tag{34}$$

Substituting (33), (25), (26) and (4) into (34) results in

$$\left(\psi^{(E1)} + \psi^{(ETC)}\right)\frac{f^{(S)}}{f^{(B)}} = \left[\psi^{(E1)}\sum_p\left(1+k_p^{(C1)}\right)\theta_p + \psi^{(ETC)} + \psi^{(B0)} + \sum_p\theta_p\psi_p^{(B1)} + k_{HEB}^{(maint)}\right] + \frac{f_{HEB}^{(maint0)}}{f^{(B)}}. \tag{35}$$

Since $\dfrac{f^{(B)}}{f^{(S)}} = \eta_{X/S}$, Eq. (35) gives the above mentioned interrelation:

$$\frac{1}{\eta_{X/S}} = \frac{1}{\eta_{X/S}^m} + \frac{f_{RO}^{(maint0)}}{f^{(B)}}, \tag{5}$$

where

$$\eta_{X/S}^m = \frac{\psi^{(E1)} + \psi^{(ETC)}}{\psi^{(E1)}\sum_p\left(1+k_p^{(C1)}\right)\theta_p + \psi^{(ETC)} + \tilde{\psi}^{(B)}}, \tag{36}$$

where $\tilde{\psi}^{(B)}$ is given by (28), and

$$f_{RO}^{(maint0)} = \frac{f_{HEB}^{(maint0)}}{\psi^{(E1)} + \psi^{(ETC)}}. \tag{37}$$

Expressions (36) and (37) are more detailed compared with (6). The energetic term in the denominator of (36), $\psi^{(E1)}\sum_p\left(1+k_p^{(C1)}\right)\theta_p + \psi^{(ETC)}$, differs from that in the numerator, $\psi^{(E1)} + \psi^{(ETC)}$. They are equivalent only when the RO interchange between the constructive and energetic metabolisms is absent as, for example, on glucose. We shall indicate below the quantities related to the growth on glucose by a superscript or a subscript "gluc". Then, $f^{(SCons\ gluc)} = f^{(Bgluc)}$, and, according to (21),

$$\sum_p\left(1+k_p^{(C1)}\right)\theta_p^{gluc} = 1. \tag{38}$$

Determination of standard constructive metabolism parameters from the data obtained during the growth on glucose

As mentioned above, the quantity $\eta_{X/S}^m$ is an estimate of maximal attainable growth yield. Parameters $\psi^{(E1)}$, $\psi^{(ETC)}$ and $k_p^{(C1)}$ present in (36) can be found from the biochemical data related to the forward metabolism and the system ETC + ATP synthase. Parameter $\tilde{\psi}^{(B)}$ can be determined by account of differences between the forward metabolism operating 1) on glucose and 2) in a given organism grown on a given substrate. The most difficult is to determine the set of θ_p since data on the relationships between biosynthetic metabolic flows are scarce. A solution of this problem is given below.

The summand $\tilde{\psi}^{(B0)}$ in (28) is related to the standard constructive metabolism and is therefore-independent of the growth substrate. Using the data for the growth on glucose, we obtain:

$$\tilde{\psi}^{(B0)} = \tilde{\psi}^{(Bgluc)} - \sum_p\theta_p\psi_p^{(B1gluc)}. \tag{39}$$

According to (38), equation (36) on glucose takes the form:

$$\eta_{X/gluc}^m = \frac{\psi^{(E1gluc)} + \psi^{(ETCgluc)}}{\psi^{(E1gluc)} + \psi^{(ETCgluc)} + \tilde{\psi}^{(Bgluc)}} \tag{40}$$

from which

$$\tilde{\psi}^{(\text{Bgluc})} = \left(\psi^{(\text{E1gluc})} + \psi^{(\text{ETCgluc})} \right) \left(1 - \frac{1}{\eta_{\text{X/gluc}}^{\text{m}}} \right). \tag{41}$$

As mentioned above, $\eta_{\text{X/gluc}}^{\text{m}} = 0.6$. For the full oxidation of glucose the GenMetPath program package gives 32 HEB's per $6O_2$ (i.e., per 24 RO), from which $\psi^{(\text{E1gluc})} + \psi^{(\text{ETCgluc})} = \frac{32}{24} = 1.333$, $\tilde{\psi}^{(\text{Bgluc})} = 0.9$.

Table 1 represents parameters of the mass-energy balance of individual pathways constituting the forward constructive metabolism during the growth on glucose. Application of Eq. (39) for finding $\tilde{\psi}^{(\text{B0})}$ requires values of the flow distribution coefficients θ_p. The latter data, firstly, are not available for the majority of published experiments in which the value $Y_{\text{X/gluc}}^{\text{m}} = 0.5$ has been obtained. Secondly, a wide occurrence of the fact that $Y_{\text{X/gluc}}^{\text{m}} = 0.5$ suggests a weak effect of θ_p variations on the value of $\sum_p \theta_p \psi_p^{(\text{B1gluc})}$.

We can rather exactly estimate only θ_p for acetyl-CoA as a precursor of lipids, based on the data that the total lipid content in cells of nonoleaginous microorganisms, as a rule, is nearly 10 % of dry biomass [Minkevich et al., 2010]. Let us denote the lipid fraction (LF) in dry biomass as LFRO if both lipids and the total biomass are measured in redoxons, and LFMass when these substances are measured in grams. Then $\text{LFRO} = \frac{\sigma_{\text{L}} \gamma_{\text{L}}}{\sigma_{\text{B}} \gamma_{\text{B}}} \text{LFMass}$, where the subscript "L" is related to lipids and "B" to the total dry cell biomass. Not all of the flows $f_p^{(\text{M})}$ arrive to a single cellular component. For example, some amino acids can be synthesized through alternative pathways beginning from different nodal metabolites. At the same time, the path acetyl-CoA $\rightarrow$ lipids can be considered to be separate.

Table 1. Parameters of mass-energy balance of glucose conversion into nodal metabolites by the forward constructive metabolism

p	Pathway	n_{HEB}	$\psi_p^{(\text{B1gluc})}$	$k_p^{(\text{C1gluc})}$
1	Glucose $\rightarrow$ 2-oxoglutarate + 4×NADH + ATP	1	−0.0625	0.5
2	Glucose + ATP $\rightarrow$ erythrose-4-phosphate + 4×NADH	−1	0.0625	0.5
3	Glucose $\rightarrow$ oxaloacetate + 7×NADH	0	0	1.4
4	Glucose + ATP $\rightarrow$ ribose-5-phosphate + 2×NADH	−1	0.05	0.2
5	Glucose $\rightarrow$ 2×3-phosphoglycerate + 2×NADH	0	0	0.2
6	Glucose $\rightarrow$ 2×phosphoenolpyruvate + 2×NADH	2	−0.1	0.2
7	Glucose $\rightarrow$ 2×pyruvate + 2×NADH +2ATP	2	−0.1	0.2
8	Glucose: growth substrate as a nodal metabolite	0	0	0
9	Glucose $\rightarrow$ 2×acetyl-CoA + 4×NADH +2ATP	4	−0.25	0.5

Redoxon molar contents:
glucose — 24, 2-oxoglutarate — 16, erythrose-4-phosphate — 16, oxaloacetate — 10,
ribose-5-phosphate — 20, 3-phosphoglycerate — 10, phosphoenolpyruvate — 10, pyruvate — 10, acetyl-CoA (without CoA) — 8, NADH (transferred reductivity units) — 2.
Phosphoenolpyruvate and acetyl-CoA are high-energy compounds.
Balance equations of the pathways (given in a shortcut form) are obtained with the GenMetPath package.
n_{HEB} is the number of high-energy bonds formed ($n_{\text{HEB}} > 0$) or consumed ($n_{\text{HEB}} < 0$) via the indicated pathways per 1 glucose molecule. The signs of n_{HEB} and $\psi_p^{(\text{B1gluc})}$ are opposite to each other.

It should be taken into account that CoA is just a carrier for acetate so that only redoxons of acetate participate in RO flows. We denote here the number p related to acetyl-CoA as p_L (in Table 1 $p_L = 9$), and the flow of RO's necessary for lipid formation from acetyl-CoA as $f_{p_L}^{(C0)}$. The ratio of the full rate of cell lipid synthesized to the rate of biomass formation is as follows:

$$\frac{f_{p_L}^{(M)} + f_{p_L}^{(C0)}}{f^{(B)}} = \text{LFRO} = \frac{\sigma_L \gamma_L}{\sigma_B \gamma_B} \text{LFMass}.$$ The overall balance of a long-chain fatty acid molecule

synthesis from acetyl-CoA catalyzed by acetyl-CoA carboxylase and fatty-acyl-CoA synthase [KEGG] is as follows: $(n + 1)$acetyl-CoA + n(ATP-ADP-P$_i$) + $2n$NADPH + $2n$H$^+$ = long-chain-acyl-CoA + nCoA + $2n$NADP$^+$, where the fatty acid molecule contains $2(n + 1)$ carbon atoms. The acetate

molecule has 8RO, one NADPH carries 2RO from which $\dfrac{f_{p_L}^{(C0)}}{f_{p_L}^{(M)}} = \dfrac{2 \times 2n}{8 \times (n+1)} \approx 0.5$ (more exactly,

a little smaller than 0.5). Then $f_{p_L}^{(M)} + f_{p_L}^{(C0)} \approx 1.5 f_{p_L}^{(M)}$ and $\theta_{p_L} = \dfrac{f_{p_L}^{(M)}}{f^{(B)}} = \dfrac{1}{1.5} \dfrac{\sigma_L \gamma_L}{\sigma_B \gamma_B} \text{LFMass}$. Here

$\sigma_B \gamma_B = 1.9$ (see above), $\sigma_L \gamma_L = 4.75$ [Minkevich et al., 2010], and $\text{LFMass} = 0.1$ from which $\theta_{p_L} = 0.167$. From Table 1 $k_{p_L}^{(C1)} = k_9^{(C1\text{gluc})} = 0.5$. Then (38) gives the following:

$$\sum_{p \neq p_L} \left(1 + k_p^{(C1\text{gluc})}\right)\theta_p = 1 - \left(1 + k_{p_L}^{(C1\text{gluc})}\right)\theta_{p_L} = 0.75. \tag{42}$$

The flow of redoxons which proceed through the pathway of lipid synthesis (through acetyl-CoA and NADPH) is $\left(1 + k_{p_L}^{(C1\text{gluc})}\right)\theta_{p_L} = 0.25$, i.e., about one quarter of the total flow $f^{(B)}$ via the constructive metabolism. The corresponding term in (39) equals $\theta_{p_L}\psi_{p_L}^{(B1\text{gluc})} = -0.167 \times 0.25 = -0.042$. Then $\tilde{\psi}^{(B0)}$ in (39) can be rewritten as follows:

$$\tilde{\psi}^{(B0)} = \tilde{\psi}^{(B\text{gluc})} - \left(\theta_{p_L}\psi_{p_L}^{(B1\text{gluc})} + u^{\text{gluc}}\right), \quad \text{where} \quad u^{\text{gluc}} = \sum_{p \neq p_L} \theta_p \psi_p^{(B1\text{gluc})}. \tag{43}$$

The remaining 3/4 of the total flow $f^{(B)}$ via the constructive metabolism are distributed over pathways responsible for the synthesis of other biomass components, and this distribution is generally unknown. Moreover, knowledge of amino acid, nucleotide and polysaccharide composition of biomass does not give information about interrelations between $f_p^{(M)}$ at $p \neq p_L$. Nevertheless, the contribution of these flows into the term u^{gluc} can be really made as follows.

Firstly let us take all terms $\left(1 + k_p^{(C1\text{gluc})}\right)\theta_p$, except that for acetyl-CoA, to be equal to each other and, hence, equal $0.75/8 = 0.094$. Denoting the values of θ_p and u^{gluc} calculated under this assumption as $\bar{\theta}_p$ and $\bar{u}^{\text{gluc}}$, we find that $\bar{\theta}_p = \dfrac{0.094}{1 + k_p^{(C1\text{gluc})}}$ and $\bar{u}^{\text{gluc}} = 0.094 \sum_{p \neq p_L} \dfrac{\psi_p^{(B1\text{gluc})}}{1 + k_p^{(C1\text{gluc})}} = -0.0118$. The values of $\bar{\theta}_p$ and $\bar{u}^{\text{gluc}}$ are given in Table 2.

Then, let us estimate the effect of possible deviations of u^{gluc} from $\bar{u}^{\text{gluc}}$ due to deviations of θ_p from $\bar{\theta}_p$. The maximal influence of θ_p on u^{gluc} takes place in the direction established by vector $\vec{\Pi} = \{\Pi_p\}$ — gradient of u^{gluc} over the *eight* variables θ_p provided that all these θ_p satisfy the equali-

ty (42). The mathematical background for the calculation of $\vec{\Pi}$ is described in **Appendix**. Components of $\vec{\Pi}$ are given in Table 2. The values of θ_p which cause a maximal deviation of u^{gluc} from $\bar{u}^{\text{gluc}}$ are calculated according to the following equation: $\theta_p = \bar{\theta}_p + k_\Pi \Pi_p$ $(p = 1 \div 8)$ where k_Π is a scale coefficient showing the degree of θ_p change along the direction of the fastest u^{gluc} alteration.

Table 2. Variations of the metabolic flow distribution, θ_p, that influence the most the high-energy bond balance in the forward constructive metabolism

p	Pathway	Π_p	$\bar{\theta}_p$	θ_p	
				$k_\Pi = -0.4$	$k_\Pi = 0.4$
1	Glucose $\rightarrow$ 2-oxoglutarate + 4×NADH	−0.0466	0.0627	0.0813	0.0440
2	Glucose $\rightarrow$ erythrose-4-phosphate + 4×NADH	0.0784	0.0627	0.0313	0.0940
3	Glucose $\rightarrow$ oxaloacetate + 7×NADH	0.0254	0.0392	0.0290	0.0493
4	Glucose $\rightarrow$ ribose-5-phosphate + 2×NADH	0.0627	0.0783	0.0533	0.1034
5	Glucose $\rightarrow$ 2×3-phosphoglycerate + 2×NADH	0.0127	0.0783	0.0733	0.0834
6	Glucose $\rightarrow$ 2×phosphoenolpyruvate + 2×NADH	−0.0873	0.0783	0.1133	0.0434
7	Glucose $\rightarrow$ 2×pyruvate + 2×NADH	−0.0873	0.0783	0.1133	0.0434
8	Glucose: growth substrate as a nodal metabolite	0.0106	0.0940	0.0898	0.0982
			$\bar{u}^{\text{gluc}} =$ $= -0.0118$	$\bar{u}^{\text{gluc}} =$ $= -0.0231$	$\bar{u}^{\text{gluc}} =$ $= -0.0004$

The vector $\vec{\Pi} = \{\Pi_p\}$ indicates the direction of the mentioned variations. For denotations see text.

It can be seen that the values $k_\Pi = \pm 0.4$ result in appreciable changes of θ_p. However, all u^{gluc} values present in Table 2 show that the total contribution of pathways proceeding via nodal metabolites No. $1 \div 8$ into the term $\sum\limits_p \theta_p \psi_p^{(\text{B1gluc})}$ (see (39)) is, by its absolute value, of the order of or notably smaller than the contribution of the "lipidic" term $\theta_{p_\text{L}} \psi_{p_\text{L}}^{(\text{B1gluc})} = -0.0157$. The difference between $\tilde{\psi}^{(\text{Bgluc})} = 0.9$ and the required value of $\tilde{\psi}^{(\text{B0})}$ can be estimated to be $\bar{\theta}_{p_\text{L}} \psi_{p_\text{L}}^{(\text{B1gluc})} + \bar{u}^{\text{gluc}} = = -0.0275 \approx -0.03$.

Hence, $\tilde{\psi}^{(\text{B0})} = 0.93$ (see (39)) can be accepted as a basic value for estimation of $\eta_{\text{X/S}}^{\text{m}}$ during the cell growth on substrates other than glucose under optimal conditions.

Another important index of the standard constructive metabolism is the ratio $\dfrac{f^{(\text{C0})}}{f^{(\text{B})}}$ which, according to (22), equals $1 - \sum\limits_p \theta_p$. We consider the standard constructive metabolism to be independent of a growth substrate. Then, using the values of θ_p found above, we obtain:

$$\frac{f^{(\text{C0})}}{f^{(\text{B})}} = 1 - \sum\limits_p \theta_p = 0.26. \tag{44}$$

It means that nearly a quarter of reductivity comes to biomass from reductivity carriers (NADPH, atc.).

Calculation of the maximal biomass energetic yield during the cell growth on substrates other than glucose

The value of biomass energy yield is expressed via the bioenergetic characteristics of the metabolism by Eq. (36). A value of $\tilde{\psi}^{(B)}$ can be found from Eq. (26) written for glucose and for a substrate of interest:

$$\tilde{\psi}^{(B\text{gluc})} = \tilde{\psi}^{(B0)} + \sum_p \theta_p \psi_p^{(B1\text{gluc})}, \quad \tilde{\psi}^{(B)} = \tilde{\psi}^{(B0)} + \sum_p \theta_p \psi_p^{(B1)}$$

from which

$$\tilde{\psi}^{(B)} = \tilde{\psi}^{(B\text{gluc})} + \sum_p \theta_p \left(\psi_p^{(B1)} - \psi_p^{(B1\text{gluc})} \right), \tag{45}$$

where $\tilde{\psi}^{(B\text{gluc})} = 0.9$. For the standard constructive metabolism the values of θ_p are the same as those for glucose, viz., $\theta_p = \overline{\theta}_p$. For $\psi_p^{(B1\text{gluc})}$ and $\overline{\theta}_p$ see Tables 1 and 2. The values of $\psi^{(E1)}$, $\psi^{(ETC)}$, $\psi_p^{(B1)}$, and $k_p^{(C1)}$ can be determined using the GenMetPath program package. Thus all the quantities necessary for application of (36) are found.

As an example, let us consider the growth on ethanol. The values of $\psi_p^{(B1)}$ and $k_p^{(C)}$ for this substrate are given in Table 3. The forward energetic metabolism of ethanol is as follows: ethanol $\rightarrow$ 5NADH + QH$_2$ + GTP; $\psi^{(E1)} = 1\text{HEB}/24\text{RO} = 0.0417$. For full oxidation of ethanol the GenMetPath program package gives 15 HEB's per 3O$_2$, from which

$$\psi^{(ETC)} + \psi^{(E1)} = 1.25, \quad \psi^{(ETC)} = 1.25 - 0.0417 \approx 1.21.$$

Then, we obtain $\eta_{X/S}^{m} = 0.54$. Using the above mentioned interrelation between energetic and mass cell yields $\left(\eta_{X/S} = \dfrac{\sigma_B \gamma_B}{\sigma_S \gamma_S} Y_{X/S} \right)$, we find $Y_{X/S}^{m} = \dfrac{\sigma_S \gamma_S}{\sigma_B \gamma_B} \eta_{X/S}^{m} = 0.89$. The yeast *Candida valida* continuously cultivated on ethanol under an optimal temperature and pH displayed $Y_{X/S}^{m} = 0.875 \pm 0.013$ [Krynitskaya et al., 1987] which is in good agreement with the estimate found here.

Effect of oxygenase and/or oxidase participation in the primary reactions of substrate metabolization

There are a number of compounds which require a direct involvement of free oxygen into the reactions that convert them into substances the further metabolization of which can be implemented through "usual" biochemical pathways. The substrates of such kind are, e.g., hydrocarbons, methanol, and, especially, compounds the structure of which has rings. A ring should be broken for subsequent oxidation of such a molecule. Enzymes catalyzing such reactions are dioxygenases (building both oxygen atoms of O$_2$ into the molecule to be oxidized), oxidases (transferring two hydrogen atoms to O$_2$ and, thus, forming hydrogen peroxide H$_2$O$_2$) and monooxygenases catalyzing reactions of a mixed type (one O atom of O$_2$ is built into the oxidized molecule and another O atom forms a water molecule H$_2$O with hydrogen atoms of the oxidized molecule).

From the viewpoint of mass-energy balance, all the mentioned reactions are essentially of the same type: they transfer four redoxons (oxygenases) or two redoxons (oxidases) of the oxidized molecule to oxygen. These redoxons annihilate with negative redoxon amount of O$_2$ due to which

Table 3. Parameters of the mass-energy balance of ethanol conversion into nodal metabolites by the constructive metabolism

p	Pathway	n_{HEB}	$\psi_p^{(B1)}$	$k_p^{(C1)}$
1	3Ethanol + ATP → 2-oxoglutarate + 10NADH	−1	0.0625	1.25
2	6Ethanol + 9ATP → 2erythrose-4-phosphate + 20NADH	−9	0.281	1.25
3	3Ethanol + 2ATP → oxaloacetate + 13NADH + GTP	−1	0.1	2.6
4	4Ethanol + 6ATP → ribose-5-phosphate + 14NADH	−6	0.3	1.4
5	2Ethanol + 2ATP → 3-phosphoglycerate + 7NADH	−2	0.2	1.4
6	2Ethanol + 2ATP → phosphoenolpyruvate + 7NADH	−1	0.1	1.4
7	2Ethanol + ATP → pyruvate + 7NADH	−1	0.1	1.4
8	4Ethanol + 6ATP → glucose + 12NADH	−6	0.25	1
9	Ethanol → acetyl-CoA + 2NADH	1	−0.125	0.5

Redoxon molar contents:
glucose — 24, 2-oxoglutarate — 16, erythrose-4-phosphate — 16, oxaloacetate — 10,
ribose-5-phosphate — 20, 3-phosphoglycerate — 10, phosphoenolpyruvate — 10, pyruvate — 10, acetyl-CoA (without CoA) — 8, NADH (transferred reductivity units) — 2.
Phosphoenolpyruvate and acetyl-CoA are high-energy compounds.
Balance equations of the pathways (given in a shortcut form) are obtained with the GenMetPath package.
n_{HEB} is the number of high-energy bonds formed ($n_{\text{HEB}} > 0$) or consumed ($n_{\text{HEB}} < 0$) via the indicated pathways per 1 glucose molecule.

organic product(s) of the reaction contain a smaller amount of RO's and, hence, less energy. The transfer of redoxons (electrons) to O_2 is mostly not coupled with high-energy bond formation. However, there are some oxidizes (e.g., methanol oxidase of methanol assimilating bacteria [Antony, 1982]) which make use of part of the electron transport chain with, correspondingly, part of the full number of coupling sites that provide ATP formation.

In the case considered here the forward constructive metabolism includes oxygenase/oxidase reactions. Calculation of $\psi_p^{(1)}$ should include ATP, GTP, etc., formed not only in reactions of so called substrate phosphorylation but also due to the possible involvement of oxygenase/oxidase reactions in redoxon injection to the electron transport chain. Let these (generally several) reactions present on the *p*-th pathway be numbered by *q*. Usually oxygenase/oxidase reactions are identical for all pathways of the preliminary constructive metabolism due to which *q*-numbers are the same for all *p*'s. We designate redoxon flows branching off the pathway from substrate to the *p*-th nodal metabolite as $f_{pq}^{(\text{OxCons})}$. Then, instead of (12), we have:

$$f_p^{(\text{SCons})} = f_p^{(M)} + f_p^{(C1)} + f_p^{(\text{OxCons})}, \quad \text{where } f_p^{(\text{OxCons})} = \sum_q f_{pq}^{(\text{OxCons})}. \tag{46}$$

The quantity $f_p^{(C1)}$ is a flow of redoxons transferred to RO carriers on the output of the forward constructive metabolism. For the full flow through this metabolism, $f^{(\text{SCons})}$, instead of (14), we have:

$$f^{(\text{SCons})} = f^{(M)} + f^{(C1)} + f^{(\text{OxCons})}, \tag{47}$$

where $$f^{(M)} = \sum_p f_p^{(M)}, \quad f^{(C1)} = \sum_p f_p^{(C1)}, \quad f^{(\text{OxCons})} = \sum_p f_p^{(\text{OxCons})} = \sum_p \sum_q f_{pq}^{(\text{OxCons})}. \tag{48}$$

Equation (47) describes sharing of substrate redoxon inflow through the constructive metabolism. It differs from (14) by the presence of an additional term $f^{(\text{OxCons})}$.

The sharing of substrate redoxon flow entering the energetic metabolism, $f^{(SEn)}$, *just after passing all the oxygenase/oxidase reactions* is as follows:

$$f^{(SEn)} = f^{(OxEn)} + f^{(E1)}, \tag{49}$$

where $f^{(OxEn)} = \sum_q f_q^{(OxEn)}$ is a sum of RO flows branched to oxygen in the course of oxygenase/oxidase operation in the energetic metabolism.

When these reactions are absent, the flow $f^{(SEn)}$ is shared according to (29), viz., mainly to the basic electron transport chain. In the presence of these enzymes the role of $f^{(SEn)}$ belongs to $f^{(E1)}$ because of which, instead of (29), a similar equation takes place:

$$f^{(ETC)} = f^{(E1)} + f^{(C1)} - f^{(C0)}. \tag{50}$$

From (49) and (50) we find the sharing of $f^{(SEn)}$:

$$f^{(SEn)} = f^{(ETC)} + f^{(OxEn)} + f^{(C0)} - f^{(C1)}. \tag{51}$$

Full redoxon balance of the whole metabolism is obtained by summation of (47) and (51) with the use of (16):

$$f^{(S)} = f^{(B)} + f^{(Ox)} + f^{(ETC)}. \tag{52}$$

Here
$$f^{(S)} = f^{(SCons)} + f^{(SEn)}, \quad f^{(Ox)} = f^{(OxCons)} + f^{(OxEn)} = \sum_q f_q^{(Ox)}, \tag{53}$$

where
$$f_q^{(Ox)} = \sum_q \left(f_q^{(OxEn)} + \sum_p f_{pq}^{(OxCons)} \right) \tag{54}$$

is the overall flow of redoxons through the q-th oxygenase/oxidase reaction. Equation (52) differs from (32) by the presence of an additional term $f^{(Ox)}$.

The balance on the level of nodal metabolites is found from (47), (51), and (50):

$$f^{(S)} = f^{(M)} + f^{(Ox)} + f^{(E1)} + f^{(C1)}. \tag{55}$$

High-energy bond balance in the case considered here includes HEB's produced due to the possible injection of redoxons into some parts of the electron transport chain by some of the oxygenase/oxidase reactions. Let $\psi_q^{(Ox)}$ be the HEB/RO ratio due to the contribution of the q-th oxygenase/oxidase reaction. Then the HEB balance is as follows:

$$\psi^{(E1)} f^{(E1)} + \psi^{(ETC)} f^{(ETC)} + \sum_q \psi_q^{(Ox)} f_q^{(Ox)} = \tilde{\psi}^{(B)} f^{(B)} + f_{HEB}^{(maint0)}. \tag{56}$$

Terms in the left-hand side of (56) describe HEB formation, those in the right-hand side of (56) are the same as earlier (biomass synthesis and cell maintenance).

Equation (56) gives the basis for finding expressions of maximal biomass yield, $\eta_{X/S}^m$, and the rate of redoxon expenditure for cell maintenance, $f_{RO}^{(maint)}$, via the bioenergetic characteristics of cell metabolism. To do this, it is necessary to transform Eq. (56) so as to retain only $f^{(S)}$, $f^{(B)}$ and $f_{HEB}^{(maint0)}$. Other rates should be eliminated using their expressions via $f^{(S)}$ and $f^{(B)}$.

Every rate of redoxon branching to oxygen in the course of a q-th oxygenase/oxidase reaction is stoichiometrically coupled to $f^{(S)}$:

$$f_q^{(Ox)} = k_q^{(Ox)} f^{(S)}. \tag{57}$$

The total rate of this branching is

$$f^{(Ox)} = \sum_q f_q^{(Ox)} = f^{(S)} \sum_q k_q^{(Ox)}. \tag{58}$$

From (52) and (58)

$$f^{(ETC)} = f^{(S)} \left(1 - \sum_q k_q^{(Ox)} \right) - f^{(B)}. \tag{59}$$

From (50), (59), (22), and (23)

$$f^{(E1)} = f^{(S)} \left(1 - \sum_q k_q^{(Ox)} \right) - f^{(B)} \sum_p \left(1 + k_p^{(C1)} \right) \theta_p. \tag{60}$$

Substituting (60), (59), and (57) into (56), taking into account that $\dfrac{f^{(S)}}{f^{(B)}} = \dfrac{1}{\eta_{X/S}}$, and using general

expressions (4)–(6), we find:

$$\eta_{X/S}^m = \frac{\psi^{(E1)} \left(1 - \sum_q k_q^{(Ox)} \right) + \psi^{(ETC)} \left(1 - \sum_q k_q^{(Ox)} \right) + \sum_q \psi_q^{(Ox)} k_q^{(Ox)}}{\psi^{(E1)} \sum_p \left(1 + k_p^{(C1)} \right) \theta_p + \psi^{(ETC)} + \tilde{\psi}^{(B)}}, \tag{61}$$

$$f_{RO}^{(maint0)} = \frac{f_{HEB}^{(maint0)}}{\psi^{(E1)} \left(1 - \sum_q k_q^{(Ox)} \right) + \psi^{(ETC)} \left(1 - \sum_q k_q^{(Ox)} \right) + \sum_q \psi_q^{(Ox)} k_q^{(Ox)}}. \tag{62}$$

In the absence of oxygenase/oxidase reactions (all $k_q^{(Ox)} = 0$) expressions (61) and (62) turn into (36) and (37).

Conclusions

Investigations of single-cell organisms (bacteria, yeasts, microscopic algae etc.) are conducted on two levels. One of them is the biochemical level, which means elucidating the details of the cellular metabolism of an organism of interest. The other consists in cultivating the cell population under pre-set conditions (temperature, pH, etc.) and measuring the physiological characteristics of the organism, e.g., the biomass yield from a substrate, which is considered in this work. Some of the mentioned experiments combine measurements of both biochemical and physiological characteristics of the organism.

Both areas of scientific investigations are of basic and applied significance. However, vast information about biochemical reactions has so far had a scanty and mostly qualitative influence on the knowledge about the physiological properties of organisms. These properties are often technological parameters in production of cell biomass or a product of cell metabolism. An *a priori* theoretical estimation of the biomass yield based on information about biochemical reactions is, thus, a rather useful approach which elucidates the possibilities of a given organism growing on a given substrate.

The novelty of the described method of such estimation consists in 1) the way by which the whole metabolism is subdivided into partial metabolisms, 2) finding the main bioenergetic stoichiometric coefficient of the standard constructive metabolism, 3) application of the GenMetPath program package for finding the bioenergetic stoichiometric coefficients of the remaining partial metabolisms,

and 4) application of the theory of mass-energy balance as a general basis for solving the considered problem.

An apparent complexity of calculations described here is caused by the complexity of the object of this investigation. The problem has been to find an optimal combination of 1) quantities computable from the stoichiometric data of substrate primary metabolization, and 2) characteristics of the whole metabolism obtained experimentally and in as general form as possible. The approach described here seems to satisfy this requirement. It makes the basis for the integration of stoichiometric parameters of numerous biochemical reactions into the biomass yield, a macroscopic index of metabolic energetic efficiency.

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Appendix. Projection of the gradient of a function on the plane, which describes a rigorous relation between the arguments

Let $u = f(\vec{x}) = f(x_1, x_2, ..., x_M)$ be a function the gradient of which is denoted here as $\vec{G} = \operatorname{grad} u$. Let

$$\vec{x}\vec{n} = D \tag{A1}$$

be the equation of a plane, which corresponds to a relation imposed upon components of $\vec{x}$. Derivative of u in the direction of unit vector $\vec{e}$ lying within the plane (1) is

$$\frac{\partial u}{\partial l} = \vec{G}\vec{e}, \qquad \left|\frac{\partial u}{\partial l}\right| = \left|\vec{G}\right|\cos\left(\widehat{\vec{G}\vec{e}}\right),$$

where $\widehat{\vec{G}\vec{e}}$ is the angle between vectors $\vec{G}$ and $\vec{e}$. The maximal directional derivative of u within the plane (A1) takes place at a minimal angle $\widehat{\vec{G}\vec{e}}$, which is the angle between $\vec{G}$ and its projection on the plane (A1), denoted below as $\vec{\Pi}$. Hence, $\vec{\Pi}$ is the projection of $\vec{G} = \operatorname{grad} u$ on the plane (A1). Vector $\vec{\Pi}$ indicates the direction of maximal variation of u value provided variables $x_1, x_2, ..., x_M$ are bound by the condition (A1).

To find the projection of vector $\vec{G}$ on the plane (A1), let us consider an arbitrary vector $\vec{V}$ the origin point of which coincides with the origin of coordinates $x_1, x_2, ..., x_M$. Then the components of $\vec{V}$ are the coordinates of its extreme point (terminus). Let us build a straight line passing through the point $\vec{V}$ perpendicularly to the plane (A1). The parametric equation of this line is

$$\vec{V}_P = \vec{V} + \alpha\vec{n}, \tag{A2}$$

where α is a parameter. Vector $\vec{n}$ is a normal to the plane (A1) (not obligatorily a unit vector), which provides a perpendicularity of the straight line to this plane. The extreme point of $\vec{V}_P$ should satisfy the equation (A1): $\vec{V}_P\vec{n} = \vec{V}\vec{n} + \alpha\left|\vec{n}\right|^2 = D$, from which

$$\alpha = \frac{D - \vec{V}\vec{n}}{\left|\vec{n}\right|^2}. \tag{A3}$$

Substitution of (A3) into (A2) results in

$$\vec{V}_P = \vec{V} + \frac{D - \left(\vec{V}\vec{n}\right)}{\left|\vec{n}\right|^2}\vec{n}. \tag{A4}$$

The vectors of our interest, $\vec{G}$ and $\vec{\Pi}$, represented by coordinates of their origins and termini, have the forms $\vec{G} = \vec{G}_2 - \vec{G}_1$ and $\vec{\Pi} = \vec{\Pi}_2 - \vec{\Pi}_1$ where indices "2" are related to the termini (extreme points)

and "1" to the vector origins. Then, from (A4) $\vec{\Pi}_1 = \vec{G}_1 + \dfrac{D - \vec{n}\vec{G}_1}{|\vec{n}|^2}\vec{n}$, $\vec{\Pi}_2 = \vec{G}_2 + \dfrac{D - \vec{n}\vec{G}_2}{|\vec{n}|^2}\vec{n}$, from which

$$\vec{\Pi} = \vec{G} - \frac{\left(\vec{n}\vec{G}\right)}{|\vec{n}|^2}\vec{n}. \tag{A5}$$

The extreme cases of interrelation (A5) are as follows. When $\vec{n} = \vec{G}$, the plane for $\vec{G}$ projection is perpendicular to $\vec{G}$; then (A5) gives $\vec{\Pi} = 0$. On the contrary, when $\vec{n} \perp \vec{G}$ ($\vec{G}$ lies within the projection plane), $\left(\vec{n}\vec{G}\right) = 0$ and $\vec{\Pi} = \vec{G}$.

In this work the function u is $u^{\text{gluc}} = \sum\limits_{p \neq p_{\text{L}}} \theta_p \psi_p^{(\text{B1gluc})}$ (see (43)), where all $\psi_p^{(\text{B1gluc})}$ are constants whereas a vector $\vec{\theta} = \{\theta_p\}$ $\left(p \neq p_{\text{L}}\right)$ plays the role of $\vec{x}$. The gradient of u^{gluc}, according to (43), equals $\vec{G} = \text{grad}_{\theta_p} u^{\text{gluc}} = \vec{\psi}_p^{(\text{B1gluc})} = \left\{\psi_1^{(\text{B1gluc})}, \psi_2^{(\text{B1gluc})}, \psi_3^{(\text{B1gluc})}, \ldots\right\}$ $\left(p \neq p_{\text{L}}\right)$. The role of restriction (A1) here belongs to the condition (42): $n_p = 1 + k_p^{(\text{C1})}$, $D = 0.68$.