КОМПЬЮТЕРНЫЕ ИССЛЕДОВАНИЯ
И МОДЕЛИРОВАНИЕ 2019 Т. 11 № 4 С. 723–750
DOI: 10.20537/2076-7633-2019-11-4-723-750

Ки&М

АНАЛИЗ И МОДЕЛИРОВАНИЕ СЛОЖНЫХ ЖИВЫХ СИСТЕМ

УДК: 519.8

Оценка максимальных значений выхода биомассы, основанная на материально-энергетическом балансе метаболизма клеток

И. Г. Минкевич

Институт биохимии и физиологии микроорганизмов им. Г. К. Скрябина РАН,
ФИЦ «Пущинский Научный центр биологических исследований РАН»,
142290, Россия, Московская область, г. Пущино, пр-т Науки, д. 5

E-mail: minkevich@ibpm.pushchino.ru

Получено 15.11.2018, после доработки — 04.06.2019.
Принято к публикации 12.07.2019.

Выход биомассы — отношение вновь синтезированного вещества растущих клеток к количеству потребленного субстрата — источника вещества и энергии для роста клеток. Выход является характеристикой эффективности конверсии субстрата в биомассу. Эта конверсия выполняется метаболизмом, который является полным множеством биохимических реакций, происходящих в клетках.

В этой работе заново рассмотрена проблема предсказания максимального выхода роста живых клеток, основанная на балансе всего метаболизма клеток и его фрагментов, названных парциальными обменами (ПО). Для рассмотрения задачи использованы следующие ПО. При росте на любом субстрате мы рассматриваем стандартный конструктивный обмен (СКО), который состоит из одинаковых метаболических путей при росте различных организмов на любом субстрате. СКО начинается с нескольких стандартных соединений (узловых метаболитов): глюкоза, ацетил-КоА, α-кетоглутарат, эритрозо-4-фосфат, оксалоацетат, рибозо-5-фосфат, 3-фосфоглицерат, фосфоенолпируват, пируват. Также рассматриваем передний метаболизм (ПМ) — остальная часть полного метаболизма. Первый ПО потребляет макроэргические связи (МЭС), образованные вторым ПО. В данной работе мы рассматриваем обобщенный вариант ПМ, когда учтены возможное наличие внеклеточных продуктов метаболизма и возможность как аэробного, так и анаэробного роста. Вместо отдельных балансов образования каждого узлового метаболита, как это было сделано в нашей предыдущей работе, данная работа имеет дело сразу со всем множеством этих метаболитов. Это делает решение задачи более компактным и требующим меньшего числа биохимических величин и значительно меньшего вычислительного времени. Выведено уравнение, выражающее максимальный выход биомассы через удельные количества МЭС, образованных и потребленных парциальными обменами. Оно содержит удельное потребление МЭС стандартным конструктивным обменом, которое является универсальным биохимическим параметром, применимым к широкому диапазону организмов и субстратов роста. Чтобы корректно определить этот параметр, полный конструктивный обмен и его передняя часть рассмотрены для роста клеток на глюкозе как наиболее изученном субстрате. Здесь мы использовали открытые ранее свойства элементного состава липидной и безлипидной частей биомассы. Было сделано численное исследование влияния вариаций соотношений между потоками через различные узловые метаболиты. Оно показало, что потребности СКО в макроэргических связях и NAD(P)H практически являются константами. Найденный коэффициент «МЭС/образованная биомасса» является эффективным средством для нахождения оценок максимального выхода биомассы из субстратов, для которых известен их первичный метаболизм. Вычисление отношения «АТФ/субстрат», необходимого для оценки выхода биомассы, сделано с помощью специального пакета компьютерных программ GenMetPath.

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

COMPUTER RESEARCH AND MODELING
2019 VOL. 11 NO. 4 P. 723–750
DOI: 10.20537/2076-7633-2019-11-4-723-750

Ки&М

ANALYSIS AND MODELING OF COMPLEX LIVING SYSTEMS

UDC: 519.8

Estimation of maximal values of biomass growth yield based on the mass-energy balance of cell metabolism

I. G. Minkevich

G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences,
Pushchino Center for Biological Research, Russian Academy of Sciences,
5 Pr. Nauki, Pushchino, Moscow Region, 142290, Russia

E-mail: minkevich@ibpm.pushchino.ru

Received 15.11.2018, after completion — 04.06.2019.
Accepted for publication 12.07.2019.

The biomass growth yield is the ratio of the newly synthesized substance of growing cells to the amount of the consumed substrate, the source of matter and energy for cell growth. The yield is a characteristic of the efficiency of substrate conversion to cell biomass. The conversion is carried out by the cell metabolism, which is a complete aggregate of biochemical reactions occurring in the cells.

This work newly considers the problem of maximal cell growth yield prediction basing on balances of the whole living cell metabolism and its fragments called as partial metabolisms (PM). The following PM's are used for the present consideration. During growth on any substrate we consider i) the standard constructive metabolism (SCM) which consists of identical pathways during growth of various organisms on any substrate. SCM starts from several standard compounds (nodal metabolites): glucose, acetyl-CoA 2-oxoglutarate, erythrose-4-phosphate, oxaloacetate, ribose-5-phosphate, 3-phosphoglycerate, phosphoenolpyruvate, and pyruvate, and ii) the full forward metabolism (FM) — the remaining part of the whole metabolism. The first one consumes high-energy bonds (HEB) formed by the second one. In this work we examine a generalized variant of the FM, when the possible presence of extracellular products, as well as the possibilities of both aerobic and anaerobic growth are taken into account. Instead of separate balances of each nodal metabolite formation as it was made in our previous work, this work deals at once with the whole aggregate of these metabolites. This makes the problem solution more compact and requiring a smaller number of biochemical quantities and substantially less computational time. An equation expressing the maximal biomass yield via specific amounts of HEB formed and consumed by the partial metabolisms has been derived. It includes the specific HEB consumption by SCM which is a universal biochemical parameter applicable to the wide range of organisms and growth substrates. To correctly determine this parameter, the full constructive metabolism and its forward part are considered for the growth of cells on glucose as the mostly studied substrate. We used here the found earlier properties of the elemental composition of lipid and lipid-free fractions of cell biomass. Numerical study of the effect of various interrelations between flows via different nodal metabolites has been made. It showed that the requirements of the SCM in high-energy bonds and NAD(P)H are practically constants. The found HEB-to-formed-biomass coefficient is an efficient tool for finding estimates of maximal biomass yield from substrates for which the primary metabolism is known. Calculation of ATP-to-substrate ratio necessary for the yield estimation has been made using the special computer program package, GenMetPath.

Keywords: biomass growth yield, cell metabolism, constructive metabolism, nodal metabolites, high-energy bonds, reductivity carriers, mass-energy balance

Citation: *Computer Research and Modeling*, 2019, vol. 11, no. 4, pp. 723–750 (Russian).

Introduction

Any system composed of a large number of constituents is characterized by macroscopic indices in which the microscopic properties of the system elements are summarized. The more structured the considered system is, the more complex is the way by which the microscopic element properties make up the features of the whole system.

The living cell metabolism represents an example of a highly structured system the elements of which are biochemical reactions. An important characteristic of the latter is its stoichiometry: a fixed distribution of the numbers of input and output compound molecules. Relevant macroscopic indices are yields of cell biomass and final products of metabolism from substrates consumed, i.e., ratios of the amount of biomass or a product to that of the metabolized substrate. Among the substrates, the source(s) of matter and energy as well as compounds participating in substrate energy transduction (e.g., oxygen) are of particular interest. Yields from such substrates are substantially dependent on growth conditions: temperature, pH (acidity or alkalinity of the growth medium), etc.

Finding the maximal value of the growth yield of microorganisms from the substrate of interest is important from both basic physiological and applied viewpoints. Experimental search for optimal growth conditions and an appropriate cultivation regime providing the maximal or close to the maximal yield is related to a high volume of laboratory work. Therefore, a topical problem is the preliminary theoretical estimation of the attainable maximal value of cell biomass yield from a given substrate.

This problem engages researchers' attention since the middle of the past century. Several ways of solving it can be marked. Some researchers propose to use parameters, which they assume to be physiological constants. For instance, Bauchop and Elsden [Bauchop, Elsden, 1960] claim the biomass yield per ATP formed in cells, Y_{ATP}, as a relatively constant quantity. This statement is rejected by Stouthamer [Stouthamer, 1979] who gives rather different values of Y_{ATP}. Another way of biomass yield prediction was proposed in [Bell, 1972]. That work was based on the comparison of experimentally measured values of biomass yield per unit of substrate reductivity (the equivalent of available electrons), $Y_{\mathrm{av\,e^-}}$, as well as yields expressed as a ratio of biomass carbon to substrate carbon, during the growth on various substrates. The author pointed out the substrate reductivity as an important factor of available values of cell yield from one substrate or another. Bell's work is a qualitative speculation and does not give any formulae for calculation of predicted yield values.

Heijnen and Roels [Heijnen, Roels, 1981] suggested the upper limit of $Y_{\mathrm{X/S}}$ (the biomass yield from substrate consumed, grams per gram) as that $Y_{\mathrm{X/S}}$ value at which the cells do not form CO_2. This means that a realistic growth of cells has to be accompanied by carbon dioxide production. This is really true for heterotrophic growth but it is only an upper $Y_{\mathrm{X/S}}$ limit, which cannot be crossed but is unclear to be achieved or not.

Recently, an area of maximal biomass yield estimation has arisen, which is based on the thermodynamic analysis, viz., the balance of Gibbs free energy [Heijnen, van Dijken, 1992; VanBriesen, Rittmann, 2000; VanBriesen, 2002; Stockar et al., 2006; Stockar et al., 2008; Xiao, VanBriesen, 2008; Stockar, 2010; Stockar et al., 2011]. A main shortcoming of this approach is just the utilization of entropy and free energy. Von Stockar points to a number of factors hampering the usage of these quantities for the analysis of living cell growth: "the daunting complexity of the biological world, characterized by giant biological molecules, multiple driving forces, heterogeneity of phases, and a multitude of biological, chemical, and physical processes occurring under rigorously irreversible conditions" [Stockar, 2010]. Nevertheless, this researcher as well as other scientists mentioned above apply the notions of entropy and Gibbs free energy to cell biomass. We consider that, in view of the above cited von Stockar's statements, the application of these quantities to living cells is still speculative, almost philosophical rather than rigorous. It is unlikely that they can be correctly calculated theoretically and unclear whether they can be measured experimentally. A thermodynamic state of any system can be identified and controlled provided that at least a partial equilibrium exists in this system. It is still un-

clear how this requirement can be satisfied. Therefore, we find all attempts to rigorously apply the notions of entropy and free energy to the substance of living cells to be insufficiently grounded.

The problem of attainable biomass yield has been considered in our previous works. The generalized elemental balance of aerobic growth, using the concept of the degree of substance reductivity, is described in [Minkevich, Eroshin, 1972]. Direct analysis of the reductivity balance during the microbial growth is given in [Minkevich, Eroshin, 1973]. In the latter work a restriction upon the cell mass yield has been derived. It follows from the restriction upon the energetic yield of cells from the substrate which cannot exceed unity. The restriction found by [Heijnen, Roels, 1981] is stronger than the latter when carbon of the substrate is reduced more that biomass carbon.

For more rigorous consideration of the cell metabolism balance a generalized unit of reductivity, redoxon (RO), has been developed [Minkevich, 1982]. This unit is based 1) on the definition of the level of zero reductivity for all chemical compounds: carbon dioxide, water ammonia orthophosphate, etc.; the physicochemical state of these substances has been rigorously defined; 2) on the amount of oxygen, which is necessary to be eliminated from substances having the zero reductivity level to form a compound of interest. For each reduced compound, its mass and reductivity (amount of RO's) are unequivocally interrelated. The properties of the redoxon are described in brief in [Minkevich et al., 2013; Minkevich, 2017]. One of these properties is the approximate constancy of the redoxon energy level in organic substances. Due to that, the yield of cell biomass from an organic substrate expressed in RO units, $\eta_{X/S}$ (RO of biomass grown/RO of substrate consumed) is quite close to the fraction of substrate energy incorporated into biomass formed. The above mentioned yield restriction obtained in [Minkevich, Eroshin, 1973] is, in terms of redoxons, as follows: $\eta_{X/S} < 1$.

However, it is clear that the realistic maximum biomass yield, expressed in any form, should be noticeably lower than that corresponding to $\eta_{X/S} = 1$. An approach for estimating attainable values of cell yield has been developed in [Minkevich, 1985]. It is based on the following considerations: 1) subdivision of the whole metabolism into three partial metabolisms: viz., constructive, respiratory and product-forming [Minkevich, Eroshin, 1976]; 2) joint consideration of redoxon and high-energy bond (HEB) balances to reveal the coupling of all the three partial metabolisms due to formation and consumption of HEB's; 3) calculation of constructive metabolism requirements in high-energy bonds based on the data concerning the biomass growth on glucose, and 4) calculation of the attainable yield on another substrate, when taking into account an effect of metabolism differences between glucose and the substrate of interest upon RO and HEB balances.

This method has given realistic predictions of biomass yield on methanol and ethanol [Minkevich, 1985]. However, it needs a general method to account for the differences between the metabolisms of glucose and the considered substrate. Foundations of such a method have been developed in our works and described in detail in [Minkevich, 2015, 2016, 2017]. As a result, the computer program package GenMetPath has been devised, which builds metabolic pathways converting a preset substrate into a preset product. The first application of GenMetPath for estimating realistic yield values is described in [Minkevich, 2017].

The present work aims to upgrade this approach. One of its goals is to reduce computational time and the number of metabolic path variants compared with that found during the separate calculation of the substrate conversion to one or another nodal metabolite. Another goal is the approach expanding to the cases of aerobic growth combined with extracellular product formation and purely anaerobic growth. The effect of varied ratios between flows via different nodal metabolites upon the cell mass-energy balance is newly estimated here.

Basics of the approach and statement of the problem

The notion of redoxon as a general unit of reductivity has been described earlier [Minkevich, 1982, 2005, 2017; Minkevich et al., 2013]. The amount of redoxons evaluates the reductivity of any substance relatively to the common zero level established by convention (CO_2, H_2O, NH_3 and others,

all in aqueous state at pH 7). The number of redoxons in 1 mole of a compound of the elemental composition $C_{n^{(C)}}H_{n^{(H)}}O_{n^{(O)}}N_{n^{(N)}}$ is $\alpha = 4n^{(C)} + n^{(H)} - 2n^{(O)} - 3n^{(N)}$ (molar reductivity).

We use here three types of metabolic flow representation: in molar units, in mass units (grams) and in terms of redoxons. The uniformity of redoxon representation is due to the fact that the absolute majority of metabolites possess nonzero reductivity, and the electron as a particle of matter also has one redoxon [Minkevich, 1982, 2013]. Distribution of the growth substrate between the biomass substance, respiratory chain, and possible organic products, as it can be seen from the equations given below, has the most simple and clear form when expressed in terms of redoxons. Therefore, the redoxon is the most convenient measure of metabolic flows related to low-molecular metabolites, biomass macromolecules and flows proceeding via the electron transport chains (ETC). Molar representation is necessary for application of GenMetPath program. The latter is not applied to cell biomass since a mole of biomass is chemically senseless. Mass representation is widely accepted for calculation of mass cell yield $Y_{X/S}$ (g of dry biomass/g of substrate).

Metabolic flows are designated here as f when measured in equivalents of redoxons, as F when measured in moles of a substance, and F^{mass} when measured in grams. Interrelations between these quantities are [Minkevich, 1982, 2005, 2017]:

$$f = \alpha F = \frac{\sigma\gamma}{12} F^{\text{mass}}. \tag{1}$$

Here

$$\alpha = 4n^{(C)} + n^{(H)} - 2n^{(O)} - 3n^{(N)} \tag{2}$$

is molar reductivity, σ is mass fraction of carbon in the substance, 12 is the atomic mass of carbon, and γ is the reduction degree of the substance carbon (the average number of redoxons per carbon atom). For an individual chemical compound, $\gamma = \alpha / n^{(C)}$. Quantities α, σ and γ are equivocally determined by the elemental composition of each substance (see above) and, therefore, are constant for every substance.

Specific carriers of reductivity, such as NAD(P) or ubiquinone Q, transfer two redoxons, which is the difference between their reduced and oxidized states. Therefore, for NAD(P)H and QH_2 the value of $\alpha = 2$:

$$f^{(\text{NAD(P)H})} = 2F^{(\text{NAD(P)})}, \quad f^{(\text{QH}_2)} = 2F^{(\text{QH}_2)}. \tag{3}$$

Free oxygen has -4 RO per one molecule [Minkevich, 1982, 2005]. Therefore, the absolute value of $f^{(\text{O}_2)}$ (the rate of oxygen consumption in RO units) is related to its molar rate as

$$\left| f^{(\text{O}_2)} \right| = 4F^{(\text{O}_2)}. \tag{4}$$

Oxygen accepts redoxons arriving to it via the main respiratory chain of cells and other reactions with direct O_2 participation so that the total rate of RO transferred to the consumed free O_2, $f^{(\text{RES})}$, equals the absolute number of oxygen redoxons:

$$f^{(\text{RES})} = \left| f^{(\text{O}_2)} \right|. \tag{5}$$

All flows considered in this work can be related to the existing dry cell substance (in this case they are the so called specific rates), or to the unit volume of the cultivation medium with cells, or to anything else. The terms “flow” and “rate” used below are synonyms.

In terms of redoxons, the yield of biomass from substrate, which is the quantity of our interest, equals

$$\eta_{X/S} = \frac{f^{(B)}}{f^{(S)}}, \tag{6}$$

where $f^{(S)}$ and $f^{(B)}$ are full flows of substrate consumption and biomass formation presented in RO units. The $\eta_{X/S}$ is, virtually, the biomass yield by energy since the energy content of one redoxon in different organic substances, including cell biomass, is approximately the same [Minkevich, 1982, 2005].

The mass yield of dry cell matter from a given substrate, $Y_{X/S}$ (a quantity widely used in microbiology and biotechnology), according to (1), is related to $\eta_{X/S}$ as follows:

$$Y_{X/S} = \frac{F^{(Bmass)}}{F^{(Smass)}} = \frac{\sigma_S \gamma_S}{\sigma_B \gamma_B} \eta_{X/S}. \quad (7)$$

As opposed to any definite chemical compound, cell biomass has a variable elemental composition depending on the organism species and cultivation conditions. Nevertheless, the quantity $\sigma_B \gamma_B$ has a rather constant value for nonoleaginous microorganisms and plant cells, viz., nearly 1.9 (for references, see [Minkevich et al., 2013; Minkevich, 2017]).

The approach developed for prediction of a maximal biomass yield value is based on the following principles.

1. Glucose is taken to be a reference growth substrate on which the maximal possible biomass yield estimated by redoxons (i.e., by energy) has been achieved. There are many cell strains which show such a yield value during their growth on glucose. We accept the stoichiometric properties of the metabolism of these organisms as basic for estimation of the expected biomass yield during the growth on other substrates.

2. Subdivision of the whole metabolism into partial metabolisms (PM). It is described in detail below. It can be made in different ways depending on the problem stated. Here we have to solve two problems.

2A. The main problem is finding the desired estimate of the attainable yield value of the given organism growing on the substrate of interest. To do this, we subdivide the whole metabolism into two parts, the forward metabolism (FM) and the standard constructive metabolism (SCM). As the border between them, we accept a set of *nodal metabolites* (this notion is introduced in [Minkevich, 2017]). The list of nodal metabolites is given below. The FM converts growth substrates into nodal metabolites as well as into a number of final products of metabolism, viz., CO_2, H_2O, possible organic extracellular products. During the aerobic growth, oxygen is also consumed in the course of the forward metabolism. So, the FM is the source of nodal metabolites as a raw material for cell biomass and of energy for the SCM to operate. This energy is delivered by high-energy bonds which are also formed by FM.

This subdivision looks like a known subdivision of the whole metabolism into catabolism and anabolism but it is not quite that. The anabolism is defined rather qualitatively, ambiguously. The SCM is a part of the anabolism which is practically the same when different organisms are grown on different substrates. The starting compounds of the SCM are defined rigorously. Besides, the list of nodal metabolites includes glucose which can simultaneously be a growth substrate. Then, a part of glucose consumed serves as a nodal metabolite and the remaining part is the substrate of the forward metabolism. If this is the case, then, from the viewpoint of catabolism and anabolism notions, it is unclear what the role of glucose is.

2B. Solution of problem 2A needs the knowledge of SCM requirements in high-energy bonds to fit the necessary energy for biomass synthesis from nodal metabolites. Moreover, our investigation described below has shown that the SCM also needs some amount of additional reductivity carried by NAD(P)H. Finding the quantities describing these requirements is another problem. It might be solved based on the complete balance of the standard constructive metabolism but this way is not realistic. On the other hand, the corresponding quantities can be determined by consideration of the forward metabolism of cells growing on glucose. This consideration allows finding the amounts of HEB and NAD(P)H necessary for SCM.

3. The balance of redoxon flows through partial metabolisms as well as through any separate biochemical reaction is subject to the law of redoxon conservation [Minkevich, 1982, 2005]. Amounts of high-energy bond formation in biochemical reactions and their aggregates are coupled with whole flows via these reactions and their sets measured both in moles and in equivalents of redoxons. All HEB formed in the cell metabolism are consumed in other parts of the metabolism. This fact establishes a stoichiometric interrelation between flows via different partial metabolisms.

The proposed list of nodal metabolites is as follows [Minkevich, 2017]: a) glucose as a source for all cellular carbohydrate synthesis; b) acetyl-CoA as a source for lipid synthesis; c) 2-oxoglutarate, erythrose-4-phosphate, oxaloacetate, ribose-5-phosphate, 3-phosphoglycerate, phosphoenolpyruvate, and pyruvate as sources of amino acid and nucleotide formation.

The balance of HEB plays a crucial role in the distribution of the redoxon flow of the substrate consumed between the SCM and other partial metabolisms. HEB formation and consumption is coupled with flows of substance measured in any units, moles, grams or redoxons; the choice of units affects only the numeric value of the coupling coefficient for any HEB-forming or -consuming process. We use here the coupling coefficients as the ratios of formed or consumed high-energy bonds per one redoxon of the flow through the considered process; letter ψ with superscripts is accepted for these coefficients. For individual reactions, quantities ψ have fixed values. For an aggregate of reactions, which includes branched flows with unfixed ratios between them, the corresponding ψ may be unfixed. The effect of this branching on the coefficient ψ for the constructive metabolism is studied below.

Alongside with HEB consumption coupled with RO flows, there exist processes of matter turnover in cells (decay and restoration of macromolecules, concentration gradients, etc.), which require high-energy bond expenditure. The overall rate of this turnover and, correspondingly, the HEB requirement is, generally, not coupled with redoxon flows via partial metabolisms, though it can contain a summand proportional to the growth rate (for references and discussion of this property of cell metabolism, see [Minkevich, 2017]). This turnover is usually called cell maintenance. The rate of HEB expenditures for cell maintenance, $F_{\mathrm{HEB}}^{(\mathrm{maint})}$, depends on the growth conditions: temperature, pH, etc. Among these factors we mark a linear dependence of $F_{\mathrm{HEB}}^{(\mathrm{maint})}$ on the growth rate of cell biomass, $f^{(\mathrm{B})}$:

$$F_{\mathrm{HEB}}^{(\mathrm{maint})} = F_{\mathrm{HEB}}^{(\mathrm{maint0})} + k_{\mathrm{HEB}}^{(\mathrm{maint})} f^{(\mathrm{B})}, \tag{8}$$

where $F_{\mathrm{HEB}}^{(\mathrm{maint0})}$ is the growth rate independent component of $F_{\mathrm{HEB}}^{(\mathrm{maint})}$ and $k_{\mathrm{HEB}}^{(\mathrm{maint})}$ the proportionality coefficient for the total HEB requirements for cell maintenance on the growth rate. Neijssel and Tempest [Neijssel, Tempest, 1976] were, probably, the first to find this dependence. Then, the effect of the biomass growth rate on cell yield from the substrate, $\eta_{\mathrm{X/S}}$, has the form (see refs in [Minkevich, 2005, 2017]):

$$\frac{1}{\eta_{\mathrm{X/S}}} = \frac{1}{\eta_{\mathrm{X/S}}^{\mathrm{m}}} + \frac{f_{\mathrm{RO}}^{(\mathrm{maint0})}}{f^{(\mathrm{B})}}. \tag{9}$$

It will be shown below that this regularity is valid for any type of cell metabolism, including that involving the formation of extracellular reduced products. Here $f_{\mathrm{RO}}^{(\mathrm{maint0})}$ is the redoxon flow through the HEB forming paths, which produce the $F_{\mathrm{HEB}}^{(\mathrm{maint0})}$ flow of high-energy bonds; $\eta_{\mathrm{X/S}}^{\mathrm{m}}$ is the so called maximal yield to which $\eta_{\mathrm{X/S}}$ tends at growth rate values much higher than $f_{\mathrm{HEB}}^{(\mathrm{maint0})}$. One of the earliest works in which the dependence of mass yield $Y_{\mathrm{X/S}}$ on the specific growth rate similar to (9) has been published is the widely known Pirt's article [Pirt, 1965].

It should be emphasized that the maximality of $\eta_{\mathrm{X/S}}^{\mathrm{m}}$ is not global with respect to all the factors affecting the cell population growth. For example, this quantity depends on cultivation temperature

and pH. We consider here $\eta_{X/S}^{m}$ as a value of the cell yield to which the $\eta_{X/S}$ tends due to $f^{(B)}$ increase *under the other conditions optimal*.

The problem of the prediction of an attainable biomass yield value includes the estimation of two quantities, $\eta_{X/S}^{m}$ and $\frac{f_{RO}^{(maint0)}}{f^{(B)}}$. At an optimal temperature and pH, both $f_{RO}^{(maint0)}$ and maximal $f^{(B)}$ depend on the organism and substrate species. For rapidly growing microbial cultures the ratio $\frac{f_{RO}^{(maint0)}}{f^{(B)}}$ is usually small compared with $\frac{1}{\eta_{X/S}^{m}}$. In any case, $\eta_{X/S}^{m}$ is the value of $\eta_{X/S}$, which cannot be exceeded by the experimentally achieved biomass yield.

Therefore, our task here is to estimate realistic values of $\eta_{X/S}^{m}$ on different substrates. For a rapidly growing organism this value will practically be an attainable maximal biomass yield. For a slowly growing one it will be an upper constraint of yield values.

In our previous work [Minkevich, 2017], the metabolic pathways forming the nodal metabolites and corresponding numbers of high energy bonds were found separately for each nodal metabolite. Besides, the problem of yield estimate was studied only for the aerobic growth of cell cultures. Here we 1) consider the balance of the metabolism for the case of simultaneously set flows via all the nodal metabolites, 2) introduce a rigorous method of selecting the constructive metabolism, and 3) generalize the form of the energetic partial metabolism including the possible excretion of reduced products. It extends to the case of purely anaerobic growth. As a result, calculations of the maximal yield become general and more efficient.

General balances of the forward and the standard constructive metabolisms

1°. *Redoxon balance of the standard constructive metabolism*. Let p be the number of a nodal metabolite, $f_p^{(M)}$ be the redoxon flow via the p-th metabolite. Formation of the totality of amino acids, nucleotides and other biomass constituents from the nodal metabolites, which is carried out by the standard constructive metabolism, requires additional reductivity, which is supplied in the form of the reduced pyridine nucleotides, NADPH and NADH. The energy levels of both carriers are identical and reductivity is reversibly exchanged between them by the enzyme transhydrogenase. Therefore, the flows of NADPH and NADH are considered here as a common, summarized flow of NAD(P)H, $f^{(N)}$, with a modified superscript. Then the total flow of redoxons coming to biomass via the standard constructive metabolism, $f^{(B)}$, equals

$$f^{(B)} = \underbrace{\sum_{p} f_p^{(M)}}_{\substack{\text{all nodal metabolites}\\ \text{consumed by SCM}}} + \underbrace{f^{(N0)}}_{\substack{\text{NAD(P)H}\\ \text{consumed by SCM}}} . \qquad (10)$$

Notation of the NAD(P)H flow on the input of the SCM, $f^{(N0)}$, contains zero in its superscript.

2°. *Redoxon balance of the forward metabolism.* Let us introduce the following notations: $f^{(S)}$ and $f^{(P)}$ are the total rates of growth substrate consumption and extracellular product formation by the FM and, hence, by the whole metabolism. Earlier we introduced $f^{(RES)}$, the rate of the redoxon flow arriving to free oxygen via all the respiratory paths of the FM including the main electron transport chain (ETC) of cells and all oxygenase and oxidase reactions not involved in this chain (see (5)). Finally, we designate the output NAD(P)H flow produced by the FM as $f^{(N1)}$. It should be emphasized that ubiquinol QH_2 is not an output compound of the FM: the complete amount of QH_2

formed within the FM by ubiquinone Q reduction is consumed by the ETC. Then the balance of the whole forward metabolism is as follows:

$$f^{(\mathrm{S})} = \underbrace{\sum_p f_p^{(\mathrm{M})}}_{\substack{\text{all nodal metabolites}\\ \text{formed by FM}}} + \underbrace{f^{(\mathrm{N1})}}_{\substack{\mathrm{NAD(P)H}\\ \text{formed by FM}}} + \underbrace{f^{(\mathrm{RES})}}_{\substack{\text{flow to oxygen}\\ \text{via all the respiratory}\\ \text{paths of FM}}} + \underbrace{f^{(\mathrm{P})}}_{\substack{\text{extracellular}\\ \text{reduced products}\\ \text{formed by FM}}}. \tag{11}$$

3°. *Redoxon balance of the full metabolism*. Since the total amount of NAD(P)H formed in the FM is utilized by the SCM,

$$f^{(\mathrm{N1})} = f^{(\mathrm{N0})}. \tag{12}$$

Elimination of $\sum_p f_p^{(\mathrm{M})}$ from (11), using (10), and utilization of (12) result in the full RO balance of the whole metabolism:

$$f^{(\mathrm{S})} = f^{(\mathrm{B})} + f^{(\mathrm{RES})} + f^{(\mathrm{P})}. \tag{13}$$

4°. *High-energy bond balance of the whole metabolism*. For every PM, the rate of high-energy bond formation or consumption, F_{HEB}, is stoichiometrically coupled with the redoxon flow via the PM, f, viz. $F_{\mathrm{HEB}} = \psi f$, where ψ is a corresponding stoichiometric coefficient. The balance of HEB forming and spending in cells based on the whole metabolism subdivision to FM and SCM is as follows:

$$F_{\mathrm{HEB}}^{(\mathrm{FM})} = F_{\mathrm{HEB}}^{(\mathrm{SCM})} + F_{\mathrm{HEB}}^{(\mathrm{maint})}. \tag{14}$$

Direct HEB expenditures for the SCM are connected with the redoxon flow via the SCM as follows:

$$F_{\mathrm{HEB}}^{(\mathrm{SCM})} = \psi^{(\mathrm{B})} f^{(\mathrm{B})}. \tag{15}$$

The right-hand side of (14), taking (8) and (15) into account, equals

$$F_{\mathrm{HEB}}^{(\mathrm{SCM})} + F_{\mathrm{HEB}}^{(\mathrm{maint0})} + k_{\mathrm{HEB}}^{(\mathrm{maint})} f^{(\mathrm{B})} = \left(\psi^{(\mathrm{B})} + k_{\mathrm{HEB}}^{(\mathrm{maint})}\right) f^{(\mathrm{B})} + F_{\mathrm{HEB}}^{(\mathrm{maint0})}. \tag{16}$$

For the FM:

$$F_{\mathrm{HEB}}^{(\mathrm{FM})} = \psi^{(\mathrm{FM})} f^{(\mathrm{S})}, \tag{17}$$

where $\psi^{(\mathrm{FM})}$ is the stoichiometric coefficient of high-energy bond formation in the course of FM operation irrespective of whether one of the flows $f^{(\mathrm{RES})}$ and $f^{(\mathrm{P})}$ or both of them are present. Then, from (14), (16), and (17):

$$\psi^{(\mathrm{FM})} f^{(\mathrm{S})} = \tilde{\psi}^{(\mathrm{B})} f^{(\mathrm{B})} + F_{\mathrm{HEB}}^{(\mathrm{maint0})}, \tag{18}$$

where

$$\tilde{\psi}^{(\mathrm{B})} = \psi^{(\mathrm{B})} + k_{\mathrm{HEB}}^{(\mathrm{maint})} \tag{19}$$

is a stoichiometric coefficient of *apparent HEB expenditures for the standard constructive metabolism*. According to this definition, $\tilde{\psi}^{(\mathrm{B})}$ differs from other analogous quantities since it includes a summand $k_{\mathrm{HEB}}^{(\mathrm{maint})}$ which is kinetic by its nature.

5°. *Cell yield expression through the stoichiometric characteristics of the PM*. Dividing (18) by $\psi^{(\mathrm{FM})} f^{(\mathrm{B})}$ and taking (6) into account, we obtain:

$$\frac{1}{\eta_{\mathrm{X/S}}} = \frac{1}{\eta_{\mathrm{X/S}}^{\mathrm{m}}} + \frac{F_{\mathrm{HEB}}^{(\mathrm{maint0})}}{\psi^{(\mathrm{FM})}} \cdot \frac{1}{f^{(\mathrm{B})}}, \tag{20}$$

where

$$\eta^{\mathrm{m}}_{\mathrm{X/S}} = \frac{\psi^{(\mathrm{FM})}}{\tilde{\psi}^{(\mathrm{B})}}. \tag{21}$$

When the values of $\psi^{(\mathrm{FM})}$ and $\tilde{\psi}^{(\mathrm{B})}$ are known, we obtain from (21) the required yield estimate. The finding of the values of $\psi^{(\mathrm{FM})}$ and $\tilde{\psi}^{(\mathrm{B})}$ is described below. These quantities characterize the energetics of the cellular metabolism. Therefore, besides their necessity for the maximal yield estimation, they are of general physiological interest per se.

6°. *Full balance of the forward metabolism*. Operation of the GenMetPath program is based on the complete balance of chemical elements and the electric charge. The basics of the GenMetPath work are described in detail in [Minkevich, 2015, 2016]. When applied to the FM, the GenMetPath considers the following process:

$$\underbrace{\mathrm{S}}_{\text{growth substrate}} + \mathrm{O_2} + \mathrm{H_3PO_4} + \mathrm{ADP} + \mathrm{NADP^+} \rightarrow \underbrace{\sum_p \mathrm{NM}_p}_{\text{all nodal metabolites formed by FM}} + \mathrm{CO_2} + \mathrm{ATP} + \mathrm{H_2O} + \mathrm{NADPH} + \mathrm{H^+} + \underbrace{\mathrm{P}}_{\text{extracellular reduced products}}, \tag{22}$$

where NM_p is the symbol of the p-th nodal metabolite. The full elemental balance of the process (22) is as follows:

$$F^{(\mathrm{S})}\mathrm{substrate} + F^{(\mathrm{O_2})}\mathrm{O_2} + F^{(\mathrm{P_i})}\mathrm{H_3PO_4} + F^{(\mathrm{ADP})}\mathrm{ADP} + F^{(\mathrm{NADP})}\mathrm{NADP^+} = \sum_p F^{(\mathrm{M})}_p \mathrm{NM}_p + F^{(\mathrm{CO_2})}\mathrm{CO_2} + F^{(\mathrm{ATP})}\mathrm{ATP} + F^{(\mathrm{H_2O})}\mathrm{H_2O} + F^{(\mathrm{NADPH})}\mathrm{NADPH} + F^{(\mathrm{H^+})}\mathrm{H^+} + F^{(\mathrm{P})}\mathrm{Product}. \tag{23}$$

The program GenMetPath finds all variants of the metabolic pathways (composed of the reactions present in its local database), which accomplish the substance conversion (23), i.e., all variants of the aggregate of flows F.

As the computational input data, the GenMetPath requires the values of all $F^{(\mathrm{M})}_p$ as well as $F^{(\mathrm{NADPH})}$ and $F^{(\mathrm{ATP})}$. All these quantities describe the output of the forward metabolism and, correspondingly, the input of the standard constructive metabolism. They are interrelated with the amount of the biomass formed by SCM, $f^{(\mathrm{B})}$. The $F^{(\mathrm{ATP})}$ and $F^{(\mathrm{NADPH})}$ describe the SCM requirements in high-energy bonds and additional reductivity. The values of $F^{(\mathrm{M})}_p$, $F^{(\mathrm{NADPH})}$, and $F^{(\mathrm{ATP})}$ should be obtained from the SCM balance (given below) which relates these flows to $f^{(\mathrm{B})}$. On the other hand, the GenMetPath calculates the remaining flows in (23), including $F^{(\mathrm{S})}$. Then. recalculation of $f^{(\mathrm{B})}$ and $F^{(\mathrm{S})}$ to the identical units allows finding the searched maximal yield in redoxon or mass units. The stoichiometric coefficient $\psi^{(\mathrm{FM})}$, which is of separate interest, equals

$$\psi^{(\mathrm{FM})} = \frac{F^{(\mathrm{ATP})}}{\alpha_{\mathrm{S}} F^{(\mathrm{S})}}. \tag{24}$$

The balance of the standard constructive metabolism

1°. Glucose is the most studied substrate on which the most stable value of the maximal yield by redoxons, i.e., by energy, has been achieved during the cultivation of various microorganisms. This

value is close to $Y_{X/S} = 0.5$ (see Table 1) and, correspondingly, $\eta_{X/S} = 0.6$. When neglecting the expenditures for cell maintenance (the specific growth rate of cells on glucose under optimal conditions is much higher than the $f^{(B)}$-independent rate of cell maintenance) we can set $\eta_{X/S}^{m\,(Gluc)} = 0.6$. The superscript "Gluc" is used here and below to designate values related to the growth on glucose. From Eqs. (14)–(16), provided that $F_{HEB}^{(maint0)} = 0$, it follows that $F_{HEB}^{(SCM\ Gluc)} = F_{HEB}^{(FM\ Gluc)}$, from which

$$\tilde{\psi}^{(B)} = \frac{F_{HEB}^{(SCM\ Gluc)}}{f^{(B\ Gluc)}} = \frac{F_{HEB}^{(FM\ Gluc)}}{f^{(B\ Gluc)}}. \tag{25}$$

It is to be recalled that the parameter $\tilde{\psi}^{(B)}$ characterizes the standard constructive metabolism and therefore is related to all or, at least, very many substrates. Nevertheless, the stability of its value is studied below.

Table 1. Experimental values of cell biomass yield obtained during cultivation of various microorganisms on media with glucose as a sole source of carbon and reductivity

Microorganism	$Y_{X/S}$	Reference
Candida utilis	0.50	Brown, Rose, 1969
Saccharomyces cerevisiae	0.50	Meyenburg, 1969
Candida utilis	0.50	Hernandez, Johnson, 1967
Candida tropicalis	0.51	Einsele et al., 1972
Escherichia coli	0.50	Morris, 1960
Escherichia coli	0.50	Ng, 1969
Pseudomonas fluorescens	0.51	Mennett, Nakayama, 1971
Arthrobacter globiformis	0.52	Morris, 1960
Escherichia coli	0.52	Ribbons, 1969

2°. As it has been told above, the quantity $\tilde{\psi}^{(B)}$ is a bioenergetic characteristic of SCM. The computational procedure of finding the $\eta_{X/S}^{m}$ estimate requires the analysis of SCM balance to obtain $F_p^{(M)}$, $F^{(NADPH)}$, and $F^{(ATP)}$. These quantities are proportional to the value of the biomass flow on the output of SCM. Therefore, the important quantities are ratios between $F_p^{(M)}$, $F^{(NADPH)}$, $F^{(ATP)}$, on one hand, and the rate of biomass growth $f^{(B)}$, on the other.

To find these ratios, we consider here another variant of the whole metabolism subdivision for the case of culture growth on glucose. Let us introduce the full constructive metabolism on glucose (CMG) which converts glucose to cell biomass. Quantitatively the CMG is composed such that the flow of glucose for biomass formation, $f^{(Scons)}$, fits the following equality:

$$f^{(Scons)} = f^{(B)}. \tag{26}$$

The flows forming the standard constructive metabolism are completely included into the CMG. At the same time, some reactions of the full forward metabolism do not participate in CMG at all whereas others provide (26) by parts of full flows via them. Operation of CMG requires high-energy bonds which are supplied by the remaining part of the set of the forward metabolism flow values.

Further, it is necessary to subdivide the full constructive metabolism on glucose into a part converting glucose into the lipid fraction of dry biomass (lipid constructive metabolism, LCM) and that transforming glucose into the remaining, lipid-free fraction (lipid-free constructive metabolism, LFCM). The grounds for this subdivision are as follows. The paths providing the synthesis of cellular

lipids proceed via acetyl-CoA as a nodal metabolite. Conversion of acetyl-CoA to lipids is rather separate from the paths transforming other nodal metabolites into other cell components. Besides, the nonoleaginous microorganisms which are the majority of microbial species have rather stable lipid content in dry cell biomass (see below). The remaining, lipid-free biomass has fairly stable characteristics of its elemental composition (also see below).

Both LCM and LFCM contain standard parts, which compose the whole SCM, and the forward parts, which are specific for glucose as a growth substrate. The latter are paths from glucose to acetyl-CoA and to all the remaining nodal metabolites, correspondingly. Below we name these forward parts with attributes "lipid" and "lipid-free".

Balances of LCM and LFCM are calculated below in the rate form. It is known that stoichiometric interrelations require the establishment of a number of basic rates to make finding the remaining rates possible. As the basic ones we accept the rates of flows via the nodal metabolites.

3°. Let us consider the balance of lipid constructive metabolism. Let $F^{(\mathrm{L\_mass})}$ be the rate of the total cell lipid synthesis expressed in mass units. The ratio of $F^{(\mathrm{L\ mass})}$ to the rate of full biomass growth, $F^{(\mathrm{B\ mass})}$, can be found using the lipid fraction in dry biomass: $\mathrm{LFMass} = \dfrac{F^{(\mathrm{L\ mass})}}{F^{(\mathrm{B\ mass})}}$. This interrelation is based on the fact that both the whole biomass and its lipid fraction are slow variables being in equilibrium with each other. The lipid content in nonoleaginous microorganisms is a relatively constant quantity: $\mathrm{LFMass} \approx 0.1$ [Minkevich et al., 2010]. The calculations below utilize an analogous quantity: the ratio of the redoxon flow arriving to lipids, $f^{(\mathrm{L})}$, to the total RO flow going to the whole biomass, $f^{(\mathrm{B})}$. Using (1), we obtain:

$$\frac{f^{(\mathrm{L})}}{f^{(\mathrm{B})}} = \kappa^{(\mathrm{L})}, \text{ where } \kappa^{(\mathrm{L})} = \frac{\sigma_{\mathrm{L}}\gamma_{\mathrm{L}}}{\sigma_{\mathrm{B}}\gamma_{\mathrm{B}}} \times \mathrm{LFMass}. \tag{27}$$

Earlier [Minkevich et al., 2010] it has been found that the quantity $\sigma_{\mathrm{L}}\gamma_{\mathrm{L}}$ has a rather constant value 4.75 (±1.8%) which is even more stable than the value of $\sigma_{\mathrm{B}}\gamma_{\mathrm{B}}$. Then $\kappa^{(\mathrm{L})} = \dfrac{4.75}{1.9} \times 0.1 = 0.25$.

The rate of lipid synthesis, $f^{(\mathrm{L})}$, includes redoxons coming to lipids both via acetyl-CoA and via NADPH. Earlier [Minkevich, 2017] we have used the following balance equation of a long-chain fatty acid molecule synthesis from acetyl-CoA catalyzed by acetyl-CoA carboxylase and fatty-acyl-CoA synthase [KEGG]:

$$\begin{aligned}&(n+1)\text{acetyl-CoA} + n(\mathrm{ATP} - \mathrm{ADP} - \mathrm{P_i}) + 2n\mathrm{NADPH} + 2n\mathrm{H^+} = \\ &\text{long-chain acyl-CoA} + n\mathrm{CoA} + 2n\mathrm{NADP^+},\end{aligned} \tag{28}$$

where the fatty acid molecule contains $2(n + 1)$ carbon atoms. Let us designate the number p corresponding to acetyl-CoA as p_{L}, the rate of acetyl-CoA's acetate group proceeding to lipids as $f_{p_{\mathrm{L}}}^{(\mathrm{M})}$, and the rate of NADPH redoxon uptake for that acetate reduction to the level of lipids as $f_{p_{\mathrm{L}}}^{(\mathrm{N0})}$. The acetate molecule has 8RO; one NADPH carries 2RO, from which $\dfrac{f_{p_{\mathrm{L}}}^{(\mathrm{N0})}}{f_{p_{\mathrm{L}}}^{(\mathrm{M})}} = \dfrac{2 \times 2n}{8 \times (n+1)} \approx 0.5$. Then $f^{(\mathrm{L})} = f_{p_{\mathrm{L}}}^{(\mathrm{M})} + f_{p_{\mathrm{L}}}^{(\mathrm{N0})} \approx 1.5 f_{p_{\mathrm{L}}}^{(\mathrm{M})}$.

An important quantity is the interrelation between the amounts of NAD(P)H formed on the path from glucose to acetyl-CoA and consumed during lipid formation from acetyl-CoA. Let $f_{p_{\mathrm{L}}}^{(\mathrm{Gluc})}$ be the rate of glucose consumption stoichiometrically related to the flow of lipid synthesis, $f^{(\mathrm{L})}$, and

let $f_{p_L}^{(N1)}$ be the rate of NADH formation during the conversion of glucose to acetyl-CoA. The stoichiometry of this process calculated by GenMetPath is as follows:

$$\begin{aligned}&\text{glucose} + 4\text{NAD}^+ + 2\text{CoA} + 2\text{ADP} + 2\text{H}_3\text{PO}_4 = \\ &2\text{Acetyl-CoA} + 4\text{NADH} + 4\text{H}^+ + 2\text{ATP} + 2\text{CO}_2 + 2\text{H}_2\text{O}.\end{aligned} \tag{29}$$

The acetate group in acetyl-CoA contains 8RO; NADH carries 2RO. Then,

$$\frac{f_{p_L}^{(N1)}}{f_{p_L}^{(M)}} = \frac{2\times 4}{8\times 2} = 0.5 \approx \frac{f_{p_L}^{(N0)}}{f_{p_L}^{(M)}}, \tag{30}$$

i.e., the amount of reductivity transferred from glucose to NAD(P)H during the conversion of glucose to acetyl-CoA can be considered as equal to the reductivity transferred from NAD(P)H to acetyl-CoA during the conversion of the latter to lipids. Therefore, the following equality is valid for the total lipid synthesis from glucose:

$$f_{p_L}^{(S)} = f^{(L)}. \tag{31}$$

Thus, conversion of glucose into lipids is not connected with NAD(P)H formation or consumption. Equation (29) will be used below in calculation of $F_{p_L}^{(M)}$ (i.e., $F^{(\text{Acetyl-CoA})}$) in the full forward metabolism balance.

4°. The remaining nodal metabolites (NM with $p \neq p_L$) are precursors for the synthesis of lipid-free biomass, i.e., the total dry cell mass without lipids. The problem is whether the conversion of these NM's into the lipid-free biomass substance requires additional reductivity and how much. The aim of this subparagraph is to find the rates of substrate consumption and reduced RO carrier formation for the requirements of the SCM provided the rates $F_p^{(M)}$ for $p \neq p_L$ are set.

Below we designate the flows related to the lipid-free constructive metabolism by letters "LF" in superscripts. Then, in accord with (23), $F^{(\text{NADPH\_LF})}$ and $F^{(\text{QH}_2\text{\_LF})}$ are the rates of NAD(P)H and QH_2 formation in the forward part of the lipid-free constructive metabolism on glucose. Instead of finding $F^{(\text{NADPH\_LF})}$ and $F^{(\text{QH}_2\text{\_LF})}$ separately, we should obtain the sum of these flows: the full forward metabolism consumes ubiquinol QH_2 and supplies an equivalent amount of NAD(P)H. Therefore, the amount of NAD(P)H redoxons at the input of the SCM equals a summarized amount of redoxons in NAD(P)H and QH_2 at the output of the FCM.

Earlier [Minkevich et al., 2010] we have discovered that the lipid-free part of dry microbial biomass has the same reduction degree of its carbon, $\gamma_{B0} = 3.967(\pm 3.3\%)$, as that of glucose, $\gamma_S = 4$. Part of the nodal metabolites (NM) has the same reduction degree (see Table 2) while the remaining NM are oxidized more than glucose and lipid-free biomass (their $\gamma_p < 4$). As a consequence of this fact, the output flows of the lipid-free FCM should include the rate of reduced carrier (NAD(P)H and QH_2) formation.

In the arrangement of nodal metabolites accepted in Table 2, $p_L = 9$. Therefore, the NMs involved in the lipid-free FCM have the numbers $p \neq 9$.

The molar balance of the lipid-free FCM is described by Eq. (23) at the following conditions: $p \neq p_L$, $F^{(P)} = 0$ (reduced products are absent), and $F^{(O_2)} = 0$ (oxygenases and oxidases do not participate in the FCM on glucose). Besides, CO_2 plays a minor role in the synthesis of amino acids and is not involved in the reactions of carbohydrate formation from glucose [Nelson, Cox, 2008]. Therefore, CO_2 is not an input compound for the lipid-free SCM and, hence, not an output compound of the lipid-free FCM, i.e., $F^{(CO_2)} = 0$.

Table 2. Reductivities and carbon contents in the nodal metabolites.
Reductivity of acetyl-CoA is related to the acetate fragment of this compound

p	Nodal metabolites	α_p, RO/mol	$n_p^{(C)}$, C/mol	γ_p, RO/C	$F_p^{(M)}$
1	2-oxoglutarate	16	5	3.2	3
2	Erythrose-4-phosphate	16	4	4	3
3	Oxaloacetate	10	4	2.5	4.8
4	Ribose-5-phosphate	20	5	4	2.4
5	3-Phosphoglycerate	10	3	3.333	4.8
6	Phosphoenolpyruvate	10	3	3.333	4.8
7	Pyruvate	10	3	3.333	4.8
8	Glucose as a nodal metabolite	24	6	4	2
9	Acetyl-CoA	8	2	4	12.6

Another peculiarity of the lipid-free FCM is that glucose is both the growth substrate and a nodal metabolite, and transition of glucose from the role of growth substrate to the role of NM is not connected with participation of other compounds, including RO and HEB carriers. Therefore, at the first part of these calculations the flow $F_8^{(M)}$ should be subtracted from the input glucose flow, $F^{(S\_LF)}$, and the left-hand side of (23) should contain a modified glucose flow:

$$F^{(S\_LF\_1)} = F^{(S\_LF)} - F_8^{(M)}. \tag{32}$$

Then Eq. (23) for the lipid-free FCM takes the following form:

$$F^{(S\_LF\_1)}\text{glucose} + F^{(P_i\_LF)}H_3PO_4 + F^{(ADP\_LF)}\text{ADP} + F^{(NADP\_LF)}\text{NADP}^+ + F^{(Q\_LF)}\text{Q} = \sum_{p=1}^{7} F_p^{(M)}\text{NM}_p + F^{(ATP\_LF)}\text{ATP} + F^{(H_2O\_LF)}H_2O + F^{(NADPH\_LF)}\text{NADPH} + F^{(H^+\_LF)}H^+ + F^{(QH_2\_LF)}QH_2. \tag{33}$$

As mentioned above, instead of separate $F^{(NADPH\_LF)}$ and $F^{(QH_2\_LF)}$, we should obtain the sum of these flows $\left(F^{(NADPH\_LF)} + F^{(QH_2\_LF)}\right)$.

The finding of $F^{(S\_LF\_1)}$ and $\left(F^{(NADPH\_LF)} + F^{(QH_2\_LF)}\right)$ requires two interrelations. These are the balances of redoxons and carbon in the lipid-free FCM, which are obtained based on the full substance balance described by Eq. (33).

The redoxon balance of the lipid-free FCM is:

$$f^{(S\_LF\_1)} = \sum_{p=1}^{7} f_p^{(M)} + f^{(NAD(P)H\_LF)} + f^{(QH_2\_LF)}. \tag{34}$$

For every redoxon-containing substance the rate in terms of RO, f, is interrelated with the corresponding molar rate, F, as $f = \alpha F$. For every carbon-containing substance the corresponding rate in gram-atoms of carbon: $F^{(carb)} = n^{(C)}F$. Hence, $F^{(carb)} = \dfrac{n^{(C)}}{\alpha} f = \dfrac{1}{\gamma} f$ where γ is the reductivity of carbon. Then, the carbon balance is as follows:

$$\frac{1}{\gamma_S} f^{(S\_LF\_1)} = \sum_{p=1}^{7} \frac{1}{\gamma_p} f_p^{(M)}. \tag{35}$$

For calculation of HEB production by the full forward metabolism using GenMetPath, the rates should be represented in a molar form. Then, Eqs. (34) and (35) take the following form:

$$\alpha_{\mathrm{S}} F^{(\mathrm{S\_LF\_1})} = \sum_{p=1}^{7} \alpha_p F_p^{(\mathrm{M})} + 2F^{(\mathrm{NAD(P)H\_LF})} + 2F^{(\mathrm{QH_2\_LF})}, \tag{36}$$

$$F^{(\mathrm{S\_LF\_1})} = \frac{1}{n_{\mathrm{S}}} \sum_{p=1}^{7} n_p F_p^{(\mathrm{M})}. \tag{37}$$

When all $F_p^{(\mathrm{M})}$ are set, $F^{(\mathrm{S\_LF1})}$ is found from (37), after which $F^{(\mathrm{NAD(P)H\_LF})} + F^{(\mathrm{QH_2\_LF})}$ can be obtained from (36). The sum $F^{(\mathrm{NAD(P)H\_LF})} + F^{(\mathrm{QH_2\_LF})}$ equals the full amount of reductivity provided by the full forward metabolism for the requirements of the lipid-free SCM, $F^{(\mathrm{NAD(P)H\ for\ LFCM})}$:

$$F^{(\mathrm{NAD(P)H\ for\ LFCM})} = F^{(\mathrm{NAD(P)H\_LF})} + F^{(\mathrm{QH_2\_LF})} = \frac{1}{2}\left(\alpha_{\mathrm{S}} F^{(\mathrm{S\_LF\_1})} - \sum_{p=1}^{7} \alpha_p F_p^{(\mathrm{M})} \right). \tag{38}$$

It is to be recalled that the flows $F^{(\mathrm{NAD(P)H\_LF})}$ and $F^{(\mathrm{QH_2\_LF})}$ are related to the lipid-free forward constructive metabolism, $F^{(\mathrm{S\_LF\_1})}$ is the rate of glucose consumption for the lipid-free constructive metabolism without glucose as a nodal metabolite, while $F^{(\mathrm{NAD(P)H\ for\ LFCM})}$ is the rate of NAD(P)H production by the full forward metabolism for the requirements of the lipid-free standard constructive metabolism.

5°. The next task is to set all $F_p^{(\mathrm{M})}$ for $p \neq 9$. Interrelations between these flows at $p = 1 \div 8$ (including $F_8^{(\mathrm{M})}$, the flow of glucose as a nodal metabolite) can vary considerably. Initially, we establish the flows via nodal metabolites in a redoxon form, $f_p^{(\mathrm{M})}$, equal to each other, which means the equal flows of energy carried to biomass by NM. After that, we shall consider the effect of variations of different $f_p^{(\mathrm{M})}$ on the value of the final quantity of interest, $\tilde{\psi}^{(\mathrm{B})}$.

Let $f_p^{(\mathrm{M})} = A$ for all $p = 1 \div 8$, where A is an arbitrary constant. Since $f_p^{(\mathrm{M})} = \alpha_p F_p^{(\mathrm{M})}$, $F_p^{(\mathrm{M})} = \dfrac{A}{\alpha_p}$. The constant A is established from the viewpoint of calculation convenience: the values of $F_p^{(\mathrm{M})}$ are desirable to be of the order of unity.

After finding $F^{(\mathrm{S\_LF\_1})}$ (Eq. (37)) and $\left(F^{(\mathrm{NAD(P)H\_LF})} + F^{(\mathrm{QH_2\_LF})}\right)$ (Eq. (38)) the full glucose amount consumed by the lipid-free FCM can be obtained using (32):

$$F^{(\mathrm{S\_LF})} = F^{(\mathrm{S\_LF\_1})} + F_8^{(\mathrm{M})} = \frac{1}{n_{\mathrm{S}}} \sum_{p=1}^{7} n_p F_p^{(\mathrm{M})} + F_8^{(\mathrm{M})}. \tag{39}$$

6°. In this subsection the quantities found above and based on the set values of $F_p^{(\mathrm{M})}$ $(p = 1 \div 8)$ are used for calculation of physiological rates necessary to obtain the SCM requirements in high-energy bonds and NAD(P)H.

To find the total rate of glucose consumption by the full forward metabolism (i.e., by the whole cell metabolism), $F^{(\mathrm{S})}$, we need to obtain the whole amount of glucose consumed by the constructive metabolism. From (26) and (31) it follows that the lipid-free part of the constructive metabolism has the same RO balance:

$$f^{(\mathrm{S\_LF})} = f^{(\mathrm{B\_LF})}. \tag{40}$$

From (26), (40), as well as interrelations $f^{(\mathrm{B})} = f^{(\mathrm{B\_LF})} + f^{(\mathrm{L})}$ and $f^{(\mathrm{L})} = \kappa^{(\mathrm{L})} f^{(\mathrm{B})}$ (see (27)), it follows:

$$f^{(\mathrm{Scons})} = \frac{f^{(\mathrm{S\_LF})}}{1-\kappa^{(\mathrm{L})}}. \tag{41}$$

The full flow of redoxons of glucose metabolized, $f^{(\mathrm{S})}$, can be found using (41) and the above mentioned value of the maximal biomass yield, $\frac{f^{(\mathrm{Scons})}}{f^{(\mathrm{S})}} = \frac{f^{(\mathrm{B})}}{f^{(\mathrm{S})}} = \eta^{\mathrm{m}}_{\mathrm{X/Glucose}} = 0.6$:

$$f^{(\mathrm{S})} = \frac{f^{(\mathrm{S\_LF})}}{\eta^{\mathrm{m}}_{\mathrm{X/Glucose}}\left(1-\kappa^{(\mathrm{L})}\right)}. \tag{42}$$

Since both flows present in (42) relate to the same compound (glucose), a similar interrelation is valid also for the corresponding molar rates:

$$F^{(\mathrm{S})} = \frac{F^{(\mathrm{S\_LF})}}{\eta^{\mathrm{m}}_{\mathrm{X/Glucose}}\left(1-\kappa^{(\mathrm{L})}\right)}, \tag{43}$$

where $F^{(\mathrm{S\_LF})}$ was found earlier (see (39)). The quantity $F^{(\mathrm{S})}$ is the total rate of glucose consumption by cells when all rates of nodal metabolite formation, $F_p^{(\mathrm{M})}$ $(p = 1 \div 8)$, are set. The flow going from glucose to lipids is taken into account by the $\kappa^{(\mathrm{L})}$-containing term.

The molar rate of oxygen consumption can be found from the redoxon balance of the growth on glucose at the RO biomass yield $\eta^{\mathrm{m}}_{\mathrm{X/Glucose}}$: $f^{(\mathrm{RES})} = \left(1-\eta^{\mathrm{m}}_{\mathrm{X/Glucose}}\right) f^{(\mathrm{S})}$ from which and from (4) and (5) as well as from $f^{(\mathrm{S})} = \alpha_{\mathrm{S}} F^{(\mathrm{S})}$ we obtain:

$$F^{(\mathrm{O_2})} = \left(1-\eta^{\mathrm{m}}_{\mathrm{X/Glucose}}\right)\frac{\alpha_{\mathrm{S}}}{4} F^{(\mathrm{S})}. \tag{44}$$

For glucose, $\frac{\alpha_{\mathrm{S}}}{4} = 6$.

The full input flow of NAD(P)H for the standard constructive metabolism equals the sum of all reductivity carriers on the output of the lipid-free forward constructive metabolism (see (38)) and that on the output of acetyl-CoA forming path (see (29)). The flow from glucose to acetyl-CoA can be found using interrelations derived above. From (27): $f^{(\mathrm{L})} = \kappa^{(\mathrm{L})} f^{(\mathrm{B})}$. Then, using (31) and (26), we obtain: $f^{(\mathrm{Gluc})}_{p_{\mathrm{L}}} = \kappa^{(\mathrm{L})} f^{(\mathrm{Scons})}$. The latter is valid also for molar flows: $F^{(\mathrm{Gluc})}_{p_{\mathrm{L}}} = \kappa^{(\mathrm{L})} F^{(\mathrm{Scons})}$. From (41) it follows that $F^{(\mathrm{Scons})} = \frac{F^{(\mathrm{S\_LF})}}{1-\kappa^{(\mathrm{L})}}$ and

$$F^{(\mathrm{Gluc})}_{p_{\mathrm{L}}} = \frac{\kappa^{(\mathrm{L})} F^{(\mathrm{S\_LF})}}{1-\kappa^{(\mathrm{L})}}. \tag{45}$$

Finally, taking into account that the amounts of NAD(P)H necessary for lipid synthesis from acetyl-CoA and formed on the path from glucose to acetyl-CoA are the same (see above), we obtain from (29):

$$F^{(\mathrm{NAD(P)H\ for\ lipid})} = 4F^{(\mathrm{Gluc})}_{p_{\mathrm{L}}} = \frac{4\kappa^{(\mathrm{L})}}{1-\kappa^{(\mathrm{L})}} F^{(\mathrm{S\_LF})}. \tag{46}$$

The total amount of NAD(P)H formed by the full forward metabolism on glucose to fit the SCM requirements, $F^{(\mathrm{N0})}$, equals the NAD(P)H amount necessary for the standard constructive metabolism on any substrate. According to (38) and (46), this quantity equals

$$F^{(\mathrm{N0})} = F^{(\mathrm{NAD(P)H\ for\ LFCM})} + F^{(\mathrm{NAD(P)H\ for\ lipid})} = \frac{1}{2}\left(\alpha_{\mathrm{S}} F^{(\mathrm{S\_LF\_1})} - \sum_{p=1}^{7} \alpha_p F_p^{(\mathrm{M})} \right) + \frac{4\kappa^{(\mathrm{L})}}{1-\kappa^{(\mathrm{L})}} F^{(\mathrm{S\_LF})}. \quad (47)$$

The last unknown quantity necessary to find $F^{(\mathrm{ATP})}$ is $F_9^{(\mathrm{M})}$, the molar rate of acetyl-CoA formation and, then, the consumption for lipid synthesis. From Eqs. (29) and (45) it follows:

$$F_9^{(\mathrm{M})} = 2F_{p_{\mathrm{L}}}^{(\mathrm{Gluc})} = \frac{2\kappa^{(\mathrm{L})} F^{(\mathrm{S\_LF})}}{1-\kappa^{(\mathrm{L})}}. \quad (48)$$

The rate of biomass growth in redoxon units, $f^{(\mathrm{B})}$, can be found from (26), (41), and (1):

$$f^{(\mathrm{B})} = f^{(\mathrm{Scons})} = \frac{f^{(\mathrm{S\_LF})}}{1-\kappa^{(\mathrm{L})}} = \frac{\alpha_{\mathrm{S}} F^{(\mathrm{S\_LF})}}{1-\kappa^{(\mathrm{L})}}, \quad (49)$$

where $F^{(\mathrm{S\_LF})}$ is taken from (39).

7°. The above described reasoning results in obtaining all the flows necessary to find the searched-for bioenergetic parameter of the standard constructive metabolism, $\tilde{\psi}^{(\mathrm{B})}$. The sequence of calculations is as follows: 1) setting the nodal metabolite flows $F_p^{(\mathrm{M})}$ for $p = 1 \div 8$; 2) calculation of the intermediary value of the glucose inflow for the lipid-free constructive metabolism, $F^{(\mathrm{S\_LF\_1})}$, from (37); 3) calculation of $F^{(\mathrm{NAD(P)H\ for\ LFCM})}$ from (38); 4) calculation of the glucose inflow for the lipid-free constructive metabolism, $F^{(\mathrm{S\_LF})}$, from (39); 5) calculation of the total glucose input flow, $F^{(\mathrm{S})}$, from (43); 6) calculation of the full rate of oxygen consumption, $F^{(\mathrm{O_2})}$, from (44); 7) calculation of the rate of acetyl-CoA synthesis for the cell lipid formation, $F_9^{(\mathrm{M})}$, from (48). After that, the rate of ATP formation in the full forward metabolism, $F^{(\mathrm{ATP})}$, can be found using the GenMetPath program, based on the stoichiometric equation of the FM:

$$\begin{aligned} &\left(F^{(\mathrm{S})} - F_8^{(\mathrm{M})}\right)\mathrm{glucose} + F^{(\mathrm{O_2})}\mathrm{O_2} + F^{(\mathrm{P_i})}\mathrm{H_3PO_4} + F^{(\mathrm{ADP})}\mathrm{ADP} + F^{(\mathrm{NADP})}\mathrm{NADP^+} = \\ &= \sum_{p\neq 8} F_p^{(\mathrm{M})}\mathrm{NM}_p + F^{(\mathrm{ATP})}\mathrm{ATP} + F^{(\mathrm{H_2O})}\mathrm{H_2O} + F^{(\mathrm{NADPH})}\mathrm{NADPH} + F^{(\mathrm{H^+})}\mathrm{H^+}. \end{aligned} \quad (50)$$

The value of $\psi^{(\mathrm{FM\ Gluc})}$ is found using (24). The searched-for value of $\tilde{\psi}^{(\mathrm{B})}$ can be obtained in two ways: from (21) or (25). The first way includes utilization of the above mentioned $\eta_{\mathrm{X/S}}^{\mathrm{m}}$ value achieved on glucose, and the second way requires application of Eq. (49) to obtain $f^{(\mathrm{B})}$.

8°. For numerical calculations we took $A = 48$. This value is arbitrary and, as mentioned above, is accepted from the viewpoint of calculation convenience. Therefore, all values of the flows F are expressed in moles per arbitrary time interval. The values of $F_p^{(\mathrm{M})}$ are given in Table 2. It is to be recalled that they correspond to the equal values of the flows via the nodal metabolites for $p = 1 \div 8$ measured in equivalents of redoxons. The equations given above result in the following quantities: $F^{(\mathrm{S\_LF\_1})} = 16.9$, $F^{(\mathrm{NAD(P)H\_LF\_FM})} = 34.8$, $F^{(\mathrm{S\_LF})} = 18.9$, $F^{(\mathrm{S})} = 42$, $F^{(\mathrm{O_2})} = 100.8$, $F_9^{(\mathrm{M})} = 12.6$, $F^{(\mathrm{NAD(P)H\ for\ SCM})} = 60$. The calculations made with the help of GenMetPath for these $F_p^{(\mathrm{M})}$ values have given 15 variants of metabolic pathways, which can be separated into two groups. Typical representatives of these groups are given in Tables 3 and 4.

Table 3. Flows via biochemical reactions involved in nodal metabolite synthesis from glucose (two typical variants, see text)

Reactions	Flows	
ETC (complex I)	201.6	167.4
ETC (remaining part)	201.6	201.6
NAD(P)+transhydrogenase (AB-specific)	–145.2	60
Hexokinase	40	40
Glucose-6-phosphate isomerase	–62.6	40
Phosphofructokinase	3.2	37.4
Fructose bisphosphate aldolase	3.2	37.4
Triosephosphate isomerase	3.2	37.4
Glyceraldehyde phosphate dehydrogenase (phosphorylating)	37.8	72
Phosphoglycerate kinase	–37.8	–72
Phosphoglycerate mutase	33	67.2
Enolase	33	67.2
Pyruvate kinase	–28.2	–62.4
Pyruvate decarboxylase	0	0
Pyruvate dehydrogenase complex	15.6	49.8
Pyruvate carboxylase	7.8	7.8
Citrate synthase	3	37.2
Aconitase (step 1)	3	37.2
Aconitase (step 2)	3	37.2
Isocitrate dehydrogenase (step 1)	3	37.2
Isocitrate dehydrogenase (step 2)	3	37.2
Oxoglutarate dehydrogenase	0	34.2
Succinyl coenzyme A synthetase (GTP)	0	–34.2
Succinate dehydrogenase	0	34.2
Fumarase (fumarate hydratase)	0	34.2
Malate dehydrogenase	0	34.2
Acetyl Co-A synthetase	0	0
Acetaldehyde dehydrogenase	0	0
Glucose-6-phosphate dehydrogenase	102.6	0
6-phosphogluconolactonase	102.6	0
Phosphogluconate dehydrogenase (decarboxylating)	102.6	0
Ribose-5-phosphate isomerase	36.8	2.6
L-Ribulose-5-phosphate 3-epimerase	65.8	–2.6
Transketolase (KEGG R01641)	34.4	0.2
Transaldolase (KEGG R08575)	34.4	0.2
Transketolase (KEGG R01067)	31.4	–2.8
CoA-independent aldehyde dehydrogenase (NAD)	0	0
Glycerol-3-phosphate 1-dehydrogenase (NADP+)	0	0
Glycerol-3-phosphate dehydrogenase (NAD+)	0	0
Glycerol-3-phosphate dehydrogenase [NAD(P)+]	0	0
ATP GDP phosphotransferase	0	–34.2

From Table 3 it can be seen that one group of the pathway variants is characterized by the absence of activity of the enzymes, which belong to the tricarboxylic acid cycle, TCA (oxoglutarate dehydrogenase and others) while the enzymes of the pentose phosphate pathway are highly active. On the other hand, another group of pathways displays an opposite pattern: a high activity of TCA enzymes. The latter is more typical of cells growing on glucose and can be considered as a biochemical basis providing the $Y_{X/S}^{m} = 0.5$ value. Table 4 shows a higher ATP formation at a high activity of TCA

reactions. The same situation takes place for all sets of $F_p^{(\mathrm{M})}$ values considered below and corresponding to deviations of $F_p^{(\mathrm{M})}$ from values given in Table 2. For the consequent analysis, we choose maximal $F^{(\mathrm{ATP})}$ values obtained for every $F_p^{(\mathrm{M})}$ set. It corresponds to maximal HEB requirements of the standard constructive metabolism. Therefore, the yield estimates obtained using these $F^{(\mathrm{ATP})}$ values will not be overrated.

Basing on these considerations, we obtained $F^{(\mathrm{ATP})} = 553$ and $\tilde{\psi}^{(\mathrm{B})} = 0.914$. Flows of the substrate consumed and biomass grown for the accepted $F_p^{(\mathrm{M})}$ are: $f^{(\mathrm{S})} = 1008$, $f^{(\mathrm{B})} = 604.8$.

Since the flows via the nodal metabolites may vary, the effect of these variations on the estimate of $\tilde{\psi}^{(\mathrm{B})}$ is of considerable interest. We have accepted values of $F_p^{(\mathrm{M})} = \frac{A}{\alpha_p} = \frac{48}{\alpha_p}$ (Table 2) as a basic variant of these flows and calculated $\tilde{\psi}^{(\mathrm{B})}$ also at modified sets of $F_p^{(\mathrm{M})}$: every $F_p^{(\mathrm{M})}$ for $p = 1 \div 8$ was multiplied by 1.5 and by 0.5 whereas the others remained equal to the basic values.

The values of $\tilde{\psi}^{(\mathrm{B})}$, obtained as described here, are present in Table 5. It should be recalled that this quantity was calculated as the amount of high-energy bonds formed by the full forward metabolism per the amount of reductivity of biomass grown. These results indicate that the requirements of the standard constructive metabolism in high-energy bond supply are practically constant. A quantity $\frac{f^{(\mathrm{NAD(P)H\ for\ SCM})}}{f^{(\mathrm{B})}}$ which is the share of biomass reductivity obtained in the form of NAD(P)H, i.e., separately from nodal metabolites, is of particular interest. The obtained values of this ratio show that, like $\tilde{\psi}^{(\mathrm{B})}$, it is also practically constant. Both quantities have the same relative variance: 3.2% of an average.

Table 4. Flows on the input and output of the full forward metabolism on glucose (two typical variants, which are the same as the variants shown in Table 3). Negative values correspond to consumption; positive, to formation of the substances

	Flows	
O_2	–100.8	–100.8
CO_2	113.4	113.4
H_2O	625.2	659.4
H^+	60	60
H_3PO_4	–534	–568.2
ATP	519	553.2
ADP	–519	–553.2
NAD(P)H	60	60
$NAD(P)^+$	–60	–60
CoA	–12.6	–12.6
Acetyl-CoA	12.6	12.6
Glucose	–40	–40
3-phosphoglycerate	4.8	4.8
Phosphoenolpyruvate	4.8	4.8
Pyruvate	4.8	4.8
Oxaloacetate	4.8	4.8
2-oxoglutarate	3	3
Ribose-5-phosphate	2.4	2.4
Erythrose-4-phosphate	3	3

Table 5. The effect of $F_p^{(\mathrm{M})}$ variations on the high-energy bond and NAD(P)H requirements for cell biomass synthesis in the course of the standard constructive metabolism. Modified flows $F_p^{(\mathrm{M})}$ are indicated by the number of corresponding nodal metabolite and by the "plus" sign when the p-th flow is increased 1.5 times, or the "minus" sign when this flow is decreased twice compared with the basic value. E.g., the basic value of $F_1^{(\mathrm{M})} = 3$ (see Table 2). The line in the present table marked by $F_{1+}^{(\mathrm{M})}$ corresponds to $F_1^{(\mathrm{M})} = 4.5$ and the line with $F_{1-}^{(\mathrm{M})}$ corresponds to $F_1^{(\mathrm{M})} = 1.5$ with the other $F_p^{(\mathrm{M})}$ unaltered

Various sets of nodal metabolite flows	$\tilde{\psi}^{(\mathrm{B})} = \dfrac{F^{(\mathrm{ATP})}}{f^{(\mathrm{B})}}$	$\dfrac{f^{(\mathrm{N0})}}{f^{(\mathrm{B})}}$
$F_p^{(\mathrm{M})}$ all basic values (see Table 2)	0.914	0.198
$F_{1+}^{(\mathrm{M})}$	0.915	0.201
$F_{1-}^{(\mathrm{M})}$	0.857	0.196
$F_{2+}^{(\mathrm{M})}$	0.857	0.193
$F_{2-}^{(\mathrm{M})}$	0.917	0.205
$F_{3+}^{(\mathrm{M})}$	0.860	0.211
$F_{3-}^{(\mathrm{M})}$	0.914	0.183
$F_{4+}^{(\mathrm{M})}$	0.857	0.193
$F_{4-}^{(\mathrm{M})}$	0.917	0.205
$F_{5+}^{(\mathrm{M})}$	0.860	0.199
$F_{5-}^{(\mathrm{M})}$	0.915	0.198
$F_{6+}^{(\mathrm{M})}$	0.860	0.199
$F_{6-}^{(\mathrm{M})}$	0.915	0.198
$F_{7+}^{(\mathrm{M})}$	0.863	0.199
$F_{7-}^{(\mathrm{M})}$	0.911	0.198
$F_{8+}^{(\mathrm{M})}$	0.859	0.193
$F_{8-}^{(\mathrm{M})}$	0.915	0.205
Average	0.888	0.198
Root-mean-square deviation	0.029	0.006
Root-mean-square deviation, % of the average	3.2	3.2

From the viewpoint of cell physiology, the constancy of both quantities present in Table 5 is an interesting fact. For the purpose of biomass yield value prediction, the constancy of $\tilde{\psi}^{(\mathrm{B})}$ is of main importance. Due to the negligible dependence of $\tilde{\psi}^{(\mathrm{B})}$ on the interrelations between $F_p^{(\mathrm{M})}$ for $p = 1 \div 8$ we shall use the values of these flows $F_p^{(\mathrm{M})} = \dfrac{A}{\alpha_p} = \dfrac{48}{\alpha_p}$ (see Table 2) when estimating maximal biomass yields from substrates other than glucose.

Calculation of the maximal biomass yield attainable under conditions other than the aerobic growth on glucose

When having the value of $\tilde{\psi}^{(\mathrm{B})}$, the maximal biomass yield from any other substrate can be obtained using Eq. (21). Recalculation of $\eta^{\mathrm{m}}_{\mathrm{X/S}}$ into conventional mass units, then, is made using (7):

$$Y^{\mathrm{m}}_{\mathrm{X/S}} = \frac{\sigma_{\mathrm{S}}\gamma_{\mathrm{S}}}{\sigma_{\mathrm{B}}\gamma_{\mathrm{B}}}\eta^{\mathrm{m}}_{\mathrm{X/S}}. \qquad (51)$$

Application of Eq. (21) requires the value of $\psi^{(\mathrm{FM})}$ characterizing the forward metabolism on this very substrate. It can be found by using the GenMetPath program. The output flow values of the forward metabolism, which are simultaneously the input values of the standard constructive metabolism, should be set for this program as those found above: all $F_p^{(\mathrm{M})}$ should be taken from Table 2, $F^{(\mathrm{NAD(P)H\ for\ LFCM})} = 34.8$, $F^{(\mathrm{ATP})} = 553$. Then, using the GenMetPath, we obtain the full flow of the substrate consumed, $F^{(\mathrm{S})}$, which then is to be recalculated to $f^{(\mathrm{S})}$ (see (1)). The value of $f^{(\mathrm{B})}$ at the mentioned $F_p^{(\mathrm{M})}$ values is the same as that found earlier for glucose: $f^{(\mathrm{B})} = 604.8$. Then, $\eta^{\mathrm{m}}_{\mathrm{X/S}} = \frac{f^{(\mathrm{B})}}{f^{(\mathrm{S})}}$.

Earlier [Minkevich, 2017] we have applied detailed analytic calculations for the forward metabolism in the case when oxygenase/oxidase reactions participate in the primary metabolization of some substrates. The technique described here makes these calculations needless: all the details of the forward metabolism are built-in in the GenMetPath.

Let us consider two examples of maximal biomass yield estimation.

For growth on ethanol as a sole source of matter and reductivity, seven variants of metabolic pathways forming the forward metabolism have been found. From the viewpoint of high-energy bond formation they can be subdivided into two groups. Four variants provide $F^{(\mathrm{ATP})} = 553$ when $f^{(\mathrm{S})} = 1108.2$ eq. RO, the remaining form the same HEB amount when $f^{(\mathrm{S})} = 1111.92$ eq. RO which are quite close to each other. Then, the estimates of maximal growth yield are: $\eta^{\mathrm{m}}_{\mathrm{X/S}} = 0.546$, $Y^{\mathrm{m}}_{\mathrm{X/S}} = 0.9$. This value is close to that obtained earlier [Minkevich, 2017]. The yeast *Candida valida* continuously cultivated on ethanol under an optimal temperature and pH displayed $Y^{\mathrm{m}}_{\mathrm{X/S}} = 0.875 \pm 0.013$ [Krynitskaya et al., 1987] which is in good agreement with the estimate found here.

Another example is the anaerobic growth of microorganisms on glucose when the forward metabolism is glucose fermentation to ethanol. Then the yield estimate is $\eta^{\mathrm{m}}_{\mathrm{X/S}} = 0.086$, $Y^{\mathrm{m}}_{\mathrm{X/S}} = 0.072$. For comparison, we take the data of Battley [Battley, 1960]. He gives the following stoichiometric equation for anaerobic growth of the yeast *Saccharomyces cerevisiae* on glucose: $C_6H_{12}O_6$ + $0.12NH_3$ = $1.54CO_2$ + 1.3ethanol + 0.43glycerol + $0{,}59CH_{1.75}O_{0.45}N_{0.20}$ (biomass). Assuming that carbon is usually about 50% of dry cell mass and taking into account that the molar mass of glucose is 180 and the atomic mass of carbon is 12, we obtain from the data of Battley: $\mathrm{biomass} = 0.59 \times 12/0.5 = 14.16$, $Y_{\mathrm{X/S}} = 14.16/180 = 0.079$. It is somewhat higher than the obtained theoretical estimate but rather close to it. The reason may be the insufficient amount of experimental data or their possible inaccuracy, though other reasons, e.g., the presence of a trace amount of oxygen, may exert some effect on the growth rate-dependent cell maintenance. This issue is in need of a special study.

Discussion

Solution of the problem of maximal biomass yield prediction has required a combined application of several techniques: mathematical analysis of metabolic flows in the redoxon, molar and mass units, and numerical calculations, which have been made using a specially developed computer program package, GenMetPath.

The important tools for the consideration given above are the notions of nodal metabolites and partial metabolisms. They were used in our previous work [Minkevich, 2017] in which the balances of high-energy bond and redoxon carriers were considered separately for each nodal metabolite. The present work operates with the aggregate of nodal metabolites as a whole. It allows us to use a smaller number of quantities necessary for this task (compared with that in [Minkevich, 2017]) and to reduce computational time by an order of magnitude (one computation procedure instead of as many as the number of nodal metabolites). The latter becomes the more important the larger the local database of compounds and reactions is. At the same time, the amounts of NAD(P)H and QH_2, which were considered earlier as a sum of reductivity carriers, i.e., as a single quantity, are considered here (during the analysis of the whole forward metabolism) as two separate quantities. This allows us to make more detailed and precise analysis of the cellular metabolism balance.

Another preference of the nodal metabolite aggregate as the output object of the forward metabolism is a realistic number of metabolic pathway variants comprising this PM. As an example, let us consider glucose as a substrate. The search for paths converting glucose separately to 2-oxoglu-tarate, erythrose-4-phosphate, oxaloacetate, ribose-5-phosphate, succinate, 3-phosphoglycerate, acetyl-CoA, phosphoenolpyruvate, and pyruvate gives the following numbers of the pathway variants: 9, 1, 15, 3, 15, 1, 3, 1, 1. Then, the number of path variants converting glucose to the whole set of the nodal metabolites is unclear. One can suppose that it may be equal to the product of these numbers, but it is 18225 which is, obviously, too much. Therefore, when considering the balances of HEB during the nodal metabolite formation, we used variants forming the maximal amounts of ATP. At the same time, conversion of glucose directly to the nodal metabolite aggregate computed by GenMetPath gives 15 variants, some of which, moreover, have been excluded as biochemically not realistic (see above).

For the basic variant of distribution between flows via the nodal metabolites (except acetyl-CoA) we accepted the case when these flows are equal to each other being expressed in redoxon units. In the present work we applied a new technique to estimate the effect of the mentioned flows inequality upon the standard constructive metabolism requirements in HEB and NAD(P)H. It has shown a notably smaller dependence of these characteristics on the NM variations than that found in our previous work (2017).

The notions of partial metabolisms are to some extent like the notions of catabolism and anabolism. Definitions of the latter given by professional biochemists are as follows. “Some pathways degrade organic nutrients into simple end products in order to extract chemical energy and convert it into a form useful to the cell; together these degradative, free-energy-yielding reactions are designated **catabolism**.” “Other pathways start with small precursor molecules and convert them to progressively larger and more complex molecules, including proteins and nucleic acids. Such synthetic pathways, which invariably require the input of energy, are collectively designated **anabolism**” [Nelson, Cox, 2008, p. 25]. These authors note that the separation of the whole metabolism into catabolism and anabolism is relative due to the presence of dual-role pathways called **amphibolic**. Our notion of partial metabolism differs from catabolism and anabolism by i) the presence of rigorous sets of the input and output substances for each growth substrate, ii) quantitative subdivision of the flows through reactions by the parts when these reactions are involved into more than one partial metabolism, and iii) the possibility to establish borders between the partial metabolisms where it is worthwhile from the viewpoint of the problem to be solved. In particular, the standard constructive metabolism differs from the anabolism by its input compounds: the nodal metabolites.

We emphasize once more that the present work considers the value of biomass yield that could be at the absence of the growth-rate-independent energy expenditures for cell maintenance. Therefore, the

whole amount of HEB formed by the forward metabolism during the culture growth on glucose has been considered to be spent for the standard constructive metabolism including its apparent needs which, in reality, are expenditures for growth-rate-dependent cell maintenance.

A notable problem is the possible variability of interrelations between flows entering the SCM through different nodal metabolites. The logic of this problem solving is as follows. A flow proceeding to cell lipids from acetyl-CoA, their main precursor, for nonoleaginous cells is approximately fixed due to the nearly constant lipid content in these cells. Flows via the remaining nodal metabolites are unknown, at least, for the organisms growing on glucose the published yield value of which is 50% by mass. Obviously, interrelations between these flows were various but resulted in the same yield value. Based on the stability of that yield value, it was found that the HEB formation was independent of these ratios within a wide range of their alteration.

Interrelation between the coefficient $\tilde{\psi}^{(\mathrm{B})}$ found in this work and the known quantity Y_{ATP} present in the literature strongly depends on the choice of nodal metabolites as border ones between the standard constructive and full forward metabolisms. The analog of Y_{ATP} in this work is $\frac{F^{(\mathrm{B\ mass})}}{F_{\mathrm{HEB}}^{(\mathrm{SCM})}} = \frac{12}{\sigma_{\mathrm{B}}\gamma_{\mathrm{B}}}\frac{f^{(\mathrm{B})}}{F_{\mathrm{HEB}}^{(\mathrm{SCM})}} = \frac{12}{\sigma_{\mathrm{B}}\gamma_{\mathrm{B}}}\frac{1}{\tilde{\psi}^{(\mathrm{B})}} = 6.9.$ This quantity is lower than the average $Y_{\mathrm{ATP}} = 10.5$ found by Bauchop and Elsden [Bauchop, Elsden, 1960] and widely accepted by many researchers. The reason for this difference is the different selection of the forward part of the whole metabolism, which provides HEB for cell biomass synthesis.

Thus, the high-energy bond expenditure for the synthesis of the standard amount of cell biomass at optimal growth conditions was found to be constant. It is the basic quantity for estimation of the maximal biomass yield on substrates other than glucose. The examples given above show the proximity of the theoretical estimates to experimental data. An important item is a correct calculation of HEB production by the forward metabolism. This task is in need of much effort if it is fulfilled manually. An efficient tool to do it represents the GenMetPath computer program package.

Abbreviations

CMG	full constructive metabolism on glucose which converts glucose to cell biomass
ETC	electron transport chain
FM	forward metabolism (full aggregate of reactions not involved in SCM)
HEB	high-energy bonds (in ATP, GTP and other specific carriers)
LCM	lipid constructive metabolism converting glucose to cell lipids
LFCM	lipid-free constructive metabolism converting glucose to the lipid-free fraction of dry cell biomass
LFMass	lipid fraction in dry biomass, g per g
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NAD(P)H	joint pool and flow of NADH and NADPH
NM	nodal metabolite
PM	partial metabolism
RO	redoxon,the generalized unit of reductivity
QH_2	ubiquinol, i.e., reduced ubiquinone
SCM	standard constructive metabolism

Nomenclature

F	general notation for the substance formation-consumption rate (flow) expressed in moles per time
$F^{(\mathrm{mass})}$	general notation for the substance formation-consumption rate (flow) expressed in grams per time
$F^{(\mathrm{Bmass})}$	full flow (rate) of biomass formation, g per time
$F_{\mathrm{HEB}}^{(\mathrm{FM})}$	rate of high-energy bond formation by the forward metabolism, moles of HEBs per time
$F^{(\mathrm{L\_mass})}$	rate of the total cell lipid synthesis, g per time
$F_p^{(\mathrm{M})}$	flow via the p-th nodal metabolite (rate of its formation and further consumption), moles per time
$F_{p_{\mathrm{L}}}^{(\mathrm{M})}$	flow via the acetyl-CoA to cellular lipids (rate of its formation and further consumption), moles per time
$F_{\mathrm{HEB}}^{(\mathrm{maint})}$	rate of high-energy bond expenditures for cell maintenance, moles of HEBs per time
$F_{\mathrm{HEB}}^{(\mathrm{maint0})}$	growth-rate-independent component of $F_{\mathrm{HEB}}^{(\mathrm{maint})}$, moles of HEBs per time
$F^{(\mathrm{NAD(P)H\_LF})}$	rate of NAD(P)H formation in the forward part of the lipid-free constructive metabolism on glucose, moles per time
$F^{(\mathrm{NAD(P)H\ for\ LFCM})}$	total rate of reductivity provided by the full forward metabolism for the requirements of the lipid-free SCM (see (38)), moles per time
$F^{(\mathrm{O_2})}$	rate of oxygen consumption, moles per time
$F^{(\mathrm{QH_2\_LF})}$	rate of QH_2 formation in the forward part of the lipid-free constructive metabolism on glucose, moles per time
$F_{\mathrm{HEB}}^{(\mathrm{SCM})}$	rate of high-energy bond consumption by the standard constructive metabolism, moles of HEBs per time
$F^{(\mathrm{S\_LF})}$	consumption rate of glucose as a growth substrate for the lipid-free biomass formation by LFCM, moles per time
$F^{(\mathrm{S\_LF})\_1}$	consumption rate of glucose as a growth substrate for the lipid-free biomass formation by LFCM minus the rate of glucose consumption for its direct utilization as a nodal metabolite (see (32)), moles per time
$F^{(\mathrm{Smass})}$	total flow (rate) of substrate consumption, g per time
Y_{ATP}	yield of cell biomass from ATP formed in cells, g per mole
$Y_{\mathrm{X/S}}$	mass cell yield, g of dry cell biomass grown per g of substrate consumed
f	general notation for the substance formation-consumption rate (flow) expressed in equivalents of redoxons (RO) per time
$f^{(\mathrm{B})}$	full flow (rate) of biomass formation, eq. RO per time
$f^{(\mathrm{L})}$	total rate of lipid synthesis, eq. RO per time

$f_p^{(\mathrm{M})}$	flow via the p-th nodal metabolite (rate of its formation and further consumption), eq. RO per time
$f_{p_\mathrm{L}}^{(\mathrm{M})}$	rate of acetyl-CoA's acetate group proceeding to lipids, eq. RO per time
$f^{(\mathrm{N0})}$	rate of NAD(P)H redoxon uptake by the standard constructive metabolism, eq. RO per time
$f_{p_\mathrm{L}}^{(\mathrm{N0})}$	rate of NAD(P)H redoxon uptake for the acetate group of acetyl-CoA reduction to the level of lipids, eq. RO per time
$f^{(\mathrm{N1})}$	total output NAD(P)H flow produced by the FM, eq. RO per time
$f_{p_\mathrm{L}}^{(\mathrm{N1})}$	flow of NADH produced by the FM for synthesis of acetyl-CoA as a precursor of cellular lipid formation, eq. RO per time
$f^{(\mathrm{O}_2)}$	rate of oxygen consumption (a negative quantity since one mole of O_2 contains –4 eq. RO), eq. RO per time
$f^{(\mathrm{P})}$	total flow (rate) of extracellular product formation by the FM and, hence, by the whole metabolism, eq. RO per time
$f^{(\mathrm{RES})}$	rate of the redoxon flow arriving to free oxygen via all the respiratory paths of the FM including the main electron transport chain of cells and all oxygenase and oxidase reactions not involved in this chain, eq. RO per time
$f^{(\mathrm{S})}$	total flow (rate) of substrate consumption by the FM and, hence, by the whole metabolism, eq. RO per time
$f^{(\mathrm{Scons})}$	flow (rate) of substrate consumption by the full constructive metabolism on glucose (glucose conversion to biomass which satisfies Eq. (26)), eq. RO per time
$f^{(\mathrm{S\_LF})}$	consumption rate of glucose as a growth substrate for the lipid-free biomass formation by LFCM, eq. RO per time
$f_{p_\mathrm{L}}^{(\mathrm{S})}$	rate of glucose consumption stoichiometrically related to the flow of lipid synthesis, $f^{(\mathrm{L})}$, eq. RO per time
$k_{\mathrm{HEB}}^{(\mathrm{maint})}$	proportionality coefficient for the total high-energy bond requirements for cell maintenance on the growth rate (see Eq. (8))
p	number of a nodal metabolite; for the list of nodal metabolites and their numbers see Table 2
p_L	number p corresponding to acetyl-CoA
α	molar reductivity of a substance (see Eq. (2))
α_p	molar reductivity of a p-th nodal metabolite
α_S	molar reductivity of a growth substrate
γ	reduction degree of a substance carbon (the average number of redoxons per carbon atom)
γ_B	reduction degree of biomass carbon
γ_{B0}	reduction degree of a lipid-free biomass carbon

γ_p	reduction degree of a *p*-th nodal metabolite carbon
γ_S	reduction degree of a growth substrate carbon
$\eta_{X/S}$	yield of cell biomass from an organic substrate, eq. RO of cells per eq. RO of substrate
$\eta_{X/S}^{m}$	maximal yield of biomass from a substrate by redoxons, i.e., the value of $\eta_{X/S}$ to which the $\eta_{X/S}$ tends at growth rate values much higher than the rate of expenditures for cell maintenance
$\kappa^{(L)}$	lipid fraction in dry cell biomass, eq. RO per eq. RO, which is simultaneously the ratio between reductivity flows going to lipids and to the whole biomass (see (27))
σ	mass fraction of carbon in a substance
σ_B	mass fraction of carbon in dry cell biomass
σ_S	mass fraction of carbon in a growth substrate
$\psi^{(B)}$	stoichiometric coefficient between HEB expenditures for the standard constructive metabolism (direct biosynthetic processes) and the biomass formation, moles of HEBs per eq. RO (see (15))
$\psi^{(FM)}$	stoichiometric coefficient between HEB formation and the substrate consumption in the full forward metabolism, moles of HEBs per eq. RO (see (17))
$\tilde{\psi}^{(B)}$	stoichiometric coefficient between apparent HEB expenditures for the standard constructive metabolism, moles of HEBs per eq. RO (see (19))

References

Bauchop T., Elsden S. R. The Growth of Micro-organisms in Relation to Their Energy Supply // Journal of General Microbiology. — 1960. — Vol. 23, No. 3. — P. 457–469.

Battley E. H. Growth Reaction Equations for *Saccharomyces cerevisiae* // Physiologia Plantarum. — 1960. — Vol. 13, No. 2. — P. 192–203.

Bell G. H. Yield Factors and Their Significance // Process Biochemistry. — 1972. — Vol. 7, No. 4. — P. 21–24, 34.

Brown C. M., Rose A. H. Effect of Temperature on Composition and Cell Volume of *Candida utilis* // Journal of Bacteriology. — 1969a. — Vol. 97, No. 1. — P. 261–272.

Brown C. M., Rose A. H. Fatty-Acid Composition of *Candida utilis* as Affected by Growth Temperature and Dissolved Oxygen Tension // Journal of Bacteriology. — 1969b. — Vol. 99, No. 2. — P. 371–378.

Einsele A., Fiechter A., Knoppel H.-P. Respiratory Activity of *Candida tropicals* during Growth on Hexadecane and Glucose // Archiv für Mikrobiologie. — 1972. — Vol. 82, No. 3. — P. 247–253.

Emel'yanova E. V., Minkevich I. G., Eroshin V. K. Growth Characteristics of the Yeast *Candida valida* VKM-Y-2327 on Glucose // Mikrobiologiya (Russian). — 1992. —Vol. 61, No. 3. — P. 422–430.

Heijnen J. J., Roels J. A. A Macroscopic Model Describing Yield and Maintenance Relationships in Aerobic Fermentation Processes // Biotechnology and Bioengineering. — 1981. — Vol. 23, No. 4. — P. 739–763.

Heijnen J. J., van Dijken J. P. In Search of a Thermodynamic Description of Biomass Yields for the Chemotrophic Growth of Microorganisms // Biotechnology and Bioengineering. — 1992. — Vol. 39, No. 8. — P. 833–858.

Hernandez E., Johnson M. J. Energy Supply and Cell Yield in Aerobically Grown Microorganisms // Journal of Bacteriology. — 1967. — Vol. 94, No. 4. — P. 996–1001.

Kleerebezem R., van Loosdrecht M. C. M. A Generalized Method for Thermodynamic State Analysis of Environmental Systems // Critical Reviews in Environmental Science and Technology. — 2010. — Vol. 40, No. 1. — P. 1–54.

Mennett R. H., Nakayama T. O. M. Influence of Temperature on Substrate and Energy Conversion in *Pseudomonas fluorescens* // Applied Microbiology. — 1971. — Vol. 22, No. 5. — P. 272–276.

Krynitskaya A. Yu., Minkevich I. G., Eroshin V. K. Study of Physiological Characteristics of the Yeast *Candida valida* VKM Y-2327 Growth in Bistat. // Applied Biochemistry and Microbiology. — 1987. — Vol. 23, No. 3. — P. 366–373 (in Russian).

von Meyenburg H. K. Energetics of the Budding Cycle of *Saccharomyces cerevisiae* during Glucose Limited Aerobic Growth // Archiv für Mikrobiologie. — 1969. — Vol. 66, No. 4. — P. 289–303.

Minkevich I. G. Physico-Chemical Properties of Organic Compounds and the Energetics of Metabolism // Journal of Theoretical Biology. — 1982. — Vol. 95, No. 3. — P. 569–590.

Minkevich I. G. Estimation of Available Efficiency of Microbial Growth on Methanol and Ethanol // Biotechnology and Bioengineering. — 1985. — Vol. 27, No. 6. — P. 792–799.

Minkevich I. G. Mass-energy balance and kinetics of the growth of microorganisms. — Moscow–Izhevsk, Regular and Chaotic Dynamics, 2005. — 351 p. (in Russian).

Minkevich I. G. Stoichiometric Synthesis of Metabolic Pathways // Computer Research and Modeling. — 2015. — Vol. 7, No. 6. — P. 1241−1267.

Minkevich I. G. Mathematical Problems of Metabolic Pathway Organization from Biochemical Reactions // Mathematical Biology and Bioinformatics. — 2016. — Vol. 11, No. 2. — P. 406–425 (in Russian).

Minkevich I. G. The Effect of Cell Metabolism on Biomass Yield during the Growth on Various Substrates // Computer Research and Modeling. — 2017. — Vol. 9, No. 6. — P. 993−1014.

Minkevich I. G., Dedyukhina E. G., Chistyakova T. I. The Effect of Lipid Content on the Elemental Composition and Energy Capacity of Yeast Biomass // Applied Microbiology and Biotechnology. — 2010. — Vol. 88, No. 3. — P. 799–806.

Minkevich I. G., Eroshin V. K. Expenditure of Oxygen and Water during Growth of Microorganisms // Izvestiya AN SSSR, seriya biologicheskaya (Proceedings of the USSR Academy of Sciences, Biological Series, Russian). — 1972. —No. 2. — P. 245–254.

Minkevich I. G., Eroshin V. K. Productivity and Heat Generation of Fermentation under Oxygen Limitation // Folia Microbiologica. — 1973. — Vol. 18, No. 5. — P. 376–385.

Minkevich I. G., Eroshin V. K. Regularities of Intracellular Mass-Energy Balance of the Growth of Microorganisms // Uspekhi Sovremennoi Biologii (Proceedings of Modern Biology, Russian). — 1976. — Vol. 82, No. 1 (4). — P. 103−116.

Minkevich I. G., Fursova P. V., Tjorlova L. D., Tsygankov A. A., Riznichenko G. Yu. The Stoichiometry and Energetics of Oxygenic Phototrophic Growth // Photosynthesis Research. — 2013. — Vol. 116, No. 1. — P. 55–78.

Morris J. G. Studies on the Metabolism of *Arthrobacter globiformis* // Journal of General Microbiology. — 1960. — Vol. 22, No. 2. — P. 564–582.

Ng H. Effect of Decreasing Growth Temperature on Cell Yield of *Escherichia coli* // Journal of Bacteriology. — 1969. — Vol. 98, No. 1. — P. 232–237.

Neijssel O. M., Tempest D. W. Bioenergetic Aspects of Aerobic Growth of *Klebsiella aerogenes* NCTC 418 in Carbon-Limited and Carbon-Sufficient Chemostat Culture // Archives of Microbiology. — 1976. — Vol. 107. — P. 215–221.

Nelson D. L., Cox M. M. Lehninger Principles of Biochemistry. — New York: W. H. Freemann and Co., 2008. — 1158 p.

Oura E. Reaction Leading to the Formation of Yeast Cell Material from Glucose and Ethanol. Appendix to the book: The Effect of Aeration on the Growth Energetics and Biochemical Composition of Baker's Yeast. — Helsinki, 1972. — P. 232–237.

Pirt S. J. The Maintenance Energy of Bacteria in Growing Cultures // Proceedings of the Royal Society. Series B. Biological Sciences. — 1965. — Vol. 163 (991). — P. 224–231.

Ribbons D. W. Automatic Assessment of Respiration during Growth in Stirred Fermentors // Applied Microbiology. — 1969. — Vol. 18, No. 3. — P. 438–443.

von Stockar U., Maskow Th., Liu J., Marison I. W., Patiño R. Thermodynamics of Microbial Growth and Metabolism: an Analysis of the Current Situation // Journal of Biotechnology. — 2006. — Vol. 121, No. 4. — P. 517–533.

von Stockar U., Vojinović V., Maskow Th., Liu J. Can Microbial Growth Yield Be Estimated Using Simple Thermodynamic Analogies To Technical Processes? // Chemical Engineering and Processing. — 2008. — Vol. 47, No. 6. — P. 980–990.

von Stockar U. Biothermodynamics of Live Cells: a Tool for Biotechnology and Biochemical Engineering // Journal of Non-Equilibrium Thermodynamics. — 2010. — Vol. 35, No. 4. — P. 415–475.

von Stockar U., Marison I., Janssen M., Patinõ R. Calorimetry and thermodynamic aspects of heterotrophic, mixotrophic, and phototrophic growth // Journal of Thermal Analysis and Calorimetry. — 2011. — Vol. 104, No. 1. — P. 45–52.

Stouthamer A. H. The Search for Correlation Between Theoretical and Experimental Growth Yields // International Review of Biochemistry. — 1979. — Vol. 21. — P. 1–47.

Stouthamer A. H. A Theoretical Study on the Amount of ATP Required for Synthesis of Microbial Cell Material // Antony van Leeuwenhoek Journal of Microbiology and Serology. — 1973. — Vol. 39, No. 3. — P. 545–565.

VanBriesen J. M. Evaluation of Methods to Predict Bacterial Yield Using Thermodynamics // Biodegradation (Kluwer Academic Publishers). — 2002. — Vol. 13, No. 3. — P. 171–190.

VanBriesen J. M., Rittmann B. E. Mathematical Description of Microbiological Reactions Involving Intermediates // Biotechnology and Bioengineering. — 2000. — Vol. 67, No. 1. — P. 35–52.

Xiao J., VanBriesen J. M. Expanded Thermodynamic True Yield Prediction Model: Adjustments and Limitations // Biodegradation. — 2008. — Vol. 19, No. 1. — P. 99–127.