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Article

Thermodynamics of Bacteria-Phage Interactions: T4 and Lambda Bacteriophages, and E. Coli Can Coexist in Natural Ecosystems due to the Ratio of their Gibbs Energies of Biosynthesis

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Abstract: The model of T4 phage, Lambda phage and Escherichia coli is often used in research on virus-host interactions. This paper reports for the first time the thermodynamic driving force of biosynthesis, catabolism and metabolism for the three organisms, on the M9 medium. Moreover, the influence of activities of nutrients and metabolic products is analyzed. All three organisms were found to have very similar Gibbs energies of metabolism. Moreover, since they share the same catabolism, their Gibbs energies of catabolism are identical. However, Gibbs energies of biosynthesis differ. The calculated thermodynamic properties have been used to explain the coexistence of both bacteria and phages in a dynamic equilibrium in natural ecosystems.

Keywords: Activity; Driving force; Metabolism; Catabolism; Environment

Introduction

Microorganisms represent biological entities, but also chemical and thermodynamic systems [von Bertalanffy, 1950, 1976; Wimmer, 2006; von Stockar, 2013a; Popovic, 2019]. As chemical and thermodynamic systems, microorganisms interact with other microorganisms and with their environment [von Bertalanffy, 1950, 1976]. Interactions between microorganisms can be analyzed quantitatively, using chemistry and thermodynamics [von Stockar, 2013a, 2013b; Ozilgen and Sorgüven, 2017; Sandler, 2017]. Energetics of interactions between microorganisms is of great importance [Mahmoudabadi et al., 2017; Yildiz and Özilgen, 2022]. During the last decades, a thermodynamic characterization has been made of bacteria [Popovic, 2019; Popovic et al., 2021], viruses [Wimmer, 2006; Molla et al., 1991; Popovic and Popovic, 2022; Popovic, 2022a, 2022b, 2022c, 2022d, 2022e, 2022f, 2022g, 2022h; Şimşek et al., 2021; Popovic and Minceva, 2021a] and phage species [Maskow et al., 2010b; Guosheng et al., 2003; Popovic and Minceva, 2020a].

Thermodynamic methods have been applied many times in life sciences. Thermodynamic analysis of organisms is as old as thermodynamics itself. The founders of thermodynamics, Lavoisier and Laplace were the first to measure heat released by a mouse using a calorimeter [Lavoisier and marquis de Laplace, 1783; Lavoisier and DeLaplace, 1994; Müller, 2010]. The first theoretical consideration was made by Boltzmann, who considered the role of entropy in energy transformations performed by organisms [Boltzmann, 1974; Popovic, 2018a, 2017]. The next was the founder of modern biothermodynamics – Schrödinger [1944], who used a thermodynamic concept, entropy, to define life. Moreover, organisms were identified as open thermodynamic systems by von Bertalanffy [1950, 1976]. Analysis of organisms became much easier due to the work of Prigogine [Glansdorff and Prigogine, 1971; Prigogine and Wiame, 1946; Prigogine, 1947, 1977; Popovic, 2018b], who developed nonequilibrium thermodynamics as a tool that proved very useful in analysis of life processes. Moreover, a great contribution to the field was given by Morowitz [Morowitz, 1995, 1992, 1968, 1955; Morowitz et al., 2000, 1988]. More recently, von Stockar analyzed the thermodynamic driving force for life processes, identifying Gibbs energy as a convenient parameter for quantitative analysis of microbial multiplication [von Stockar and Liu, 1999; von Stockar, 2013a, 2013b; von Stockar et al., 2006; Patiño et al., 2007; Von Stockar and Marison, 1993; Liu et al., 2001]. Hansen discussed change in information content and entropy in organisms during life processes, including evolution [Hansen et al., 2021, 2018, 2009]. Lucia discussed the importance of entropy and Gibbs energy in life sciences [Lucia and Grisolia, 2020; Lucia, 2015], and sustainability and economy [Lucia et al., 2022; Grisolia et al., 2022; Lucia and Grisolia, 2021]. Calorimetric measurements and biothermodynamic analysis of microorganisms have been performed by Maskow [Maskow et al., 2010a; Maskow and Paufler, 2015; Maskow and Harms, 2006; Maskow and von Stockar, 2005; Maskow, 2013; Korth et al., 2021; Fricke et al., 2019]. Thermodynamics has been applied to the fields of soil research by Barros [Barros, 2021; Barros et al., 2021, 2020, 2016; Xu et al., 2017]. Therefore, thermodynamics has been in use in biological research for over 200 years and has given many interesting results [Berg et al., 2002].

Thermodynamics has been applied to research on viruses. Gale made thermodynamic analysis of virus-host interactions during virus attachment and entry into the host cell [Gale, 2022, 2021, 2020, 2019, 2018]. Lucia applied thermodynamics in studies on virology and epidemiology [Lucia et al., 2021, 2020a, 2020b; Kaniadakis et al., 2020]. Maskow et al. [2010b] used calorimetry to study transitioning of viruses from lysogenic into lytic cycle. Guosheng et al. [2003] made a calorimetric study of the bacteriophage lytic life cycle.

Calorimetry has been used to study viruses, since the accurate methods became available 50 years ago [Privalov, 2013; Sarge et al., 2018]. Recent calorimetric studies of bacteriophages include: capsid stability [Bauer et al., 2015; Bauer and Evilevitch, 2015], infectivity [Bauer and Evilevitch, 2015; Li et al., 2015; Evilevitch, 2018], structure [Bauer et al., 2015; Bauer and Evilevitch, 2015; Li et al., 2015; Evilevitch, 2018; Chakraborty et al., 2012], preservation [Petsong et al., 2021; Chang et al., 2019; Zhang et al., 2020; Leung et al., 2016; Malik et al., 2021], interaction with bacteria [Petsong et al., 2021; Lerchner et al., 2011; Morais et al., 2014; Tkhilaishvili et al., 2018; Wang et al., 2020], effect against biofilms [Tkhilaishvili et al., 2020a, 2020b, 2018a, 2018b, 2018c; Morais et al., 2014; Mariana, 2015], capsid disassembly [Qiu, 2012], medical applications [Chang et al., 2020, 2019; Boggione et al., 2021; Zhang et al., 2020; Leung et al., 2016; Heselpoth et al., 2015; Malik et al., 2021], transition from lysogenic to lytic cycle [Maskow et al., 2013b; Xu et al., 2018], viral enzyme studies [Kurochkina et al., 2012; Lee et al., 2017; Plotka et al., 2015] and application in biosensors [Lee et al., 2019]. The methods used for calorimetric studies of bacteriophages include isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC) and reaction calorimetry (a.k.a. isothermal microcalorimetry) [Privalov, 2013; Sarge et al., 2018].

Bacteria and phages interact at two main sites: at the cell membrane and in the cytoplasm. At the cell membrane, the phages attach to the host cells and enter the cytoplasm. In the cytoplasm, the phages multiply. In both processes, energetics plays an important role [Gale, 2022, 2021, 2020, 2019, 2018; Popovic and Minceva, 2020b; Popovic and Popovic, 2022; Popovic, 2022a, 2022b].

Thermodynamic properties of viruses can be determined based on their elemental composition, using predictive models developed by Patel and Ericson [1981] and Battley [1999, 1998, 1992]. These methods are very important, since most thermodynamic laboratories lack the required biosafety level for virus analysis [Popovic, 2022c]. To enable calculation of thermodynamic properties from virus elemental composition, the atom counting method was suggested [Popovic, 2022c]. The atom counting method was found to give results in good agreement with available experimental data [Popovic, 2022c; Wimmer, 2006].

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The goal of this paper is to calculate and compare the thermodynamic driving forces for multiplication of T4 and Lambda bacteriophages, and their host Escherichia coli. The analysis was performed on the M9 medium, since it is widely used and chemically well characterized. The thermodynamic driving force is quantified by Gibbs energy [von Stockar, 2013b; von Stockar and Liu, 1999], which was determined for biosynthesis, catabolism and metabolism as a whole, for each analyzed organism. Moreover, the influence of activities of nutrients and products of microbial growth were taken into consideration.

Methods

Based on the literature data on elemental composition and standard thermodynamic properties of the T4 phage, Lambda phage, E. coli live matter and M9 growth medium, a thermodynamic analysis was made of the interactions between the organisms. This section describes the data sources and the approach used for the analysis.

Data sources

Elemental compositions of T4 and Lambda bacteriophages were taken from [Popovic and Minceva, 2020a]. Elemental composition of the host E. coli was taken from [Battley, 1992; Bauer and Ziv, 1976]. Elemental compositions of the analyzed organisms are given in Table 1.

Table 1: Elemental composition of T4 and Lambda bacteriophages, and their host Escherichia coli. The amount of element *J* in the empirical formula of live matter is denoted with n_J.

Organism	n_C	n_H	n_O	n_N	n_P	n_S	Reference
T4 phage	1	1.4445	0.4167	0.3120	0.0398	0.0032	Popovic and Minceva, 2020a
Lambda phage	1	1.4174	0.4470	0.3271	0.0511	0.0031	Popovic and Minceva, 2020a
E. coli	1	1.77	0.49	0.24	0	0	Bauer and Ziv, 1976
E. coli	1	1.74	0.464	0.26	0	0	Battley, 1992

Chemical composition of the M9 growth medium supplemented with glucose is given in Table 2. The concentrations of the nutrients were taken from [Geerlof, 2010; Cold Spring Harbor Protocols, 2022]. The oxygen concentration was taken from [Xing et al., 2014]. Standard thermodynamic properties of the medium components were taken from [Atkins and de Paula, 2011; 2014; Rard and Wolery, 2007]. Ionic radii were taken from [Kielland, 1937; Skoog et al., 2013].

Table 2: Composition of the growth medium. The microorganisms were cultured in an M9 medium with glucose. For every substance, *C* denotes molarity, a_{ion} the ionic radius (given for charged species only), *z* charge in elementary units, γ_{DH} the activity coefficient calculated using the extended Debye–Hückel equation and a_{DH} activity calculated using the extended Debye–Hückel equation. The activity of glucose was set to one, because its thermodynamic properties were calculated as described in the methods section. The activity of live matter (Bio) was set to one, since it represents a distinct phase from the solution. The activity of water was set to one, since it is the solvent.

Substance	C (mol/dm³)	a_{ion} (cm)	z (q_e)	γ_{DH}	a_{DH}
$C_6H_{12}O_6$ (aq)	0.00002397	N.A.	0	1	1
NH_4^+ (aq)	0.00935	3.00E-08	+1	0.722876	0.006759
HPO_4^{2-} (aq)	0.0337	4.00E-08	-2	0.304436	0.01026
SO_4^{2-} (aq)	0.001	4.00E-08	-2	0.304436	0.000304
O_2 (g)	2.56E-04	N.A.	0	1	0.000256
Bio	1	N.A.	0	1	1
$H_2PO_4^-$ (aq)	0.022	4.00E-08	-1	0.742804	0.016342
H_2O (l)	1	N.A.	0	1	1
Na^+ (aq)	0.07595	4.00E-08	+1	0.742804	0.056416
K^+ (aq)	0.022	3.00E-08	+1	0.722876	0.015903

Cl^- (aq)	0.0185	3.00E-08	-1	0.722876	0.013373
Mg^{2+} (aq)	0.001	8.00E-08	+2	0.410357	0.00041
Ca^{2+} (aq)	0.0003	6.00E-08	+2	0.361107	0.000108
CO_2 (aq)	1.29E-05	N.A.	0	1	1.29E-05

Standard thermodynamic properties of the analyzed organisms were taken from [Popovic and Minceva, 2020a; Popovic, 2019], including standard enthalpy of formation, $\Delta_f H^0(Bio)$, standard molar entropy, $S_m{}^0(Bio)$, and standard Gibbs energy of formation, $\Delta_f G^0(Bio)$. They are given in Table 3.

Table 3: Standard thermodynamic properties of live matter of the T4 and Lambda bacteriophages, and their host Escherichia coli. The values were taken from [Popovic, 2019; Popovic and Minceva, 2020a]. $\Delta_f H^0(Bio)$ represents standard enthalpy of formation of live matter (relative to elements). $\Delta_f H^0(Bio)$ was calculated using the Patel-Erickson equation [Patel and Erickson, 1981]. $S_m{}^0(Bio)$ denotes standard molar entropy of live matter. $S_m{}^0(Bio)$ was calculated using the Battley equation [Battley, 1999]. $\Delta_f G^0(Bio)$ represents standard Gibbs energy of formation of live matter (relative to elements). $\Delta_f G^0(Bio)$ was calculated by combining $\Delta_f H^0(Bio)$ and $S_m{}^0(Bio)$. .

Organism	$\Delta_f H^0$(Bio) (kJ/C-mol)	$S_m{}^0$(Bio) (J/C-mol K)	$\Delta_f G^0$(Bio) (kJ/C-mol)
T4 phage	-94.4	32.6	-52.2
Lambda phage	-102.5	33.2	-59.5
E. coli	-114.1	36.4	-67.0
E. coli	-107.4	35.8	-60.9

Stoichiometry and standard thermodynamic properties of biosynthesis

Elemental composition of the analyzed microorganisms was used to construct their biosynthesis half-reactions, macrochemical equations describing conversion of nutrients into new live matter. Elemental composition of live matter can be used to find stoichiometric coefficients in the biosynthesis half-reaction [Von Stockar, 2013b; Sandler, 2017]. This is done through stoichiometry, using conservation of matter and charge [Von Stockar, 2013b; Sandler, 2017]. The stoichiometric coefficients of the biosynthesis half-reactions were calculated using the formulas in Table 4.

Table 4: Stoichiometric coefficients for the biosynthesis half-reactions on the M9 growth medium. The general growth reaction on the M9 growth medium is $C_6H_{12}O_6 + NH_4^+ + HPO_4^{2-} + SO_4^{2-} + O_2 \rightarrow (Bio) + H_2PO_4^- + H_2O$. Stoichiometric coefficients of all substances in the biosynthesis half-reactions can be determined from the elemental composition of live matter, using the formulas from this table. The values of stoichiometric coefficients of products are positive, while those of the reactants are negative.

Nutrient	Stoichiometric coefficient for biosynthesis half-reaction
$C_6H_{12}O_6$ (aq)	$- n_C/6$
NH_4^+ (aq)	$- n_N$
HPO_4^{2-} (aq)	$n_P + 2\ n_S - n_N$
SO_4^{2-} (aq)	$- n_S$
O_2 (g)	$- ¼\ (2\ n_O + 3\ n_N - n_H - 5\ n_P - 6\ n_S)$
Bio	+1
$H_2PO_4^-$ (aq)	$n_N - 2\ n_P - 2\ n_S$
H_2O (l)	$[½\ (2\ n_O + 3\ n_N - n_H - 5\ n_P - 6\ n_S) + n_C + 4\ n_P + 4\ n_S - n_O]$

Standard thermodynamic properties of the biosynthesis half-reaction include standard enthalpy of biosynthesis, $\Delta_{bs}H^0$, standard entropy of biosynthesis, $\Delta_{bs}S^0$, and standard Gibbs energy of biosynthesis, $\Delta_{bs}G^0$. Standard thermodynamic properties of biosynthesis are at standard conditions: standard temperature of 298.15 K (25°C) and pressure 1 bar, as well as unit activities of all reactants and products [Atkins and de Paula, 2011, 2014]. The values of standard thermodynamic properties of biosynthesis were calculated applying the Hess's law to the biosynthesis half-reactions.

$$\Delta_r H^0 = \sum_i \nu_i \, \Delta_f H^0(i) \tag{1}$$

$$\Delta_r S^0 = \sum_i \nu_i \, S_m^0(i) \tag{2}$$

$$\Delta_r G^0 = \sum_i \nu_i \, \Delta_f G^0(i) \tag{3}$$

where $\Delta_r H^0$ denotes standard reaction enthalpy, $\Delta_r S^0$ standard reaction entropy, $\Delta_r G^0$ standard reaction Gibbs energy, ν_i is the stoichiometric coefficient of substance i, $\Delta_f H^0(i)$ standard enthalpy of formation of substance i, $S_m^0(i)$ standard molar entropy of substance i and $\Delta_f G^0(i)$ standard Gibbs energy of formation of substance i [Atkins and de Paula, 2011, 2014].

Stoichiometry and thermodynamic properties of catabolism

The energy source for all the analyzed organisms is glucose and their metabolism is aerobic. Thus, the catabolic half-reaction represents aerobic oxidation of glucose: $C_6H_{12}O_6 + 6\ O_2 = 6\ CO_2 + 6\ H_2O$. The stoichiometric coefficients of the catabolic half-reaction were substituted into the Hess's law (equations 1-3) to find standard thermodynamic properties of catabolism, including standard enthalpy of catabolism, $\Delta_{cat}H^0$, standard entropy of catabolism, $\Delta_{cat}S^0$, and standard Gibbs energy of catabolism, $\Delta_{cat}G^0$. Since viruses hijack the metabolic machinery of their host cells, they share the same catabolism [Popovic, 2022i]. Thus, the catabolic half-reaction is identical for all the analyzed microorganisms [Popovic, 2022i].

Driving force of growth and biomass yield

The catabolic and biosynthesis half-reactions are combined into the growth reaction for an organism, describing the organism's entire metabolism [von Stockar, 2013a, 2013b; Heijnen, 2013]. The Gibbs energy change of the growth reaction is Gibbs energy of growth (metabolism), also known as the driving force of growth [von Stockar, 2013a, 2013b; von Stockar and Liu, 1999]. Gibbs energy of growth of heterotrophic organisms depends only on the carbon and energy source, and not on the nature of catabolic reaction and the electron acceptor used [Heijnen, 2013]. Moreover, Gibbs energy of growth can be determined from the properties of the carbon and energy source.

Heijnen and van Dijken [1992] found that the standard Gibbs energy of growth (metabolism), $\Delta_{met}G^0$, is given by the equation

$$\begin{aligned} -\Delta_{met}G^0\left(\frac{kJ}{C-mol}\right) &= 200 + 18 \cdot \left(6 - n_{C,s}\right)^{1.8} \\ &+ \exp\left\{\left[(3.8 - \Gamma_s)^2\right]^{0.16} \cdot \left(3.6 + 0.4\, n_{C,s}\right)\right\} \end{aligned} \tag{4a}$$

Where $n_{C,s}$ is the number of carbon atoms in the substrate molecule (for glucose $n_{C,s} = 6$) while Γ_s denotes the degree of reduction of the substrate molecules [Heijnen and van Dijken, 1992]. The generalized degree of reduction of organic substance i, Γ_i, is given by the equation

$$\Gamma_i = \frac{4\, n_{C,i} + n_{H,i} - 2\, n_{O,i}}{n_{C,i}} \tag{4b}$$

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Where $n_{C,i}$, $n_{H,i}$ and $n_{O,i}$ are the numbers of C, H and O atoms in the formula of the substance, respectively [Sandler, 2017]. Equation (4a) was developed empirically by considering growth yields of many microorganisms on various substrates [Heijnen and van Dijken, 1992]. The explanation is that there is an optimum substrate degree of reduction, which is similar to that of the live matter [Heijnen and van Dijken, 1992; Heijnen, 2013]. At this degree of reduction, Gibbs energy required to drive the metabolism is minimal, since less changes need to be made to the substrate to incorporate it into the organism's live matter [Heijnen and van Dijken, 1992; Heijnen, 2013]. Going away from the optimal degree of reduction means that more Gibbs energy has to be dissipated [Heijnen and van Dijken, 1992; Heijnen, 2013].

A similar equation was proposed by Liu et al. [2007]

$$\Delta_{met}G^0\left(\frac{kJ}{C-mol}\right) = \frac{666.2}{\Gamma_s} + 243.1 \qquad \text{for } \Gamma_s \leq 4.67 \qquad (5a)$$

$$\Delta_{met}G^0\left(\frac{kJ}{C-mol}\right) = 157 \cdot \Gamma_s - 339 \qquad \text{for } \Gamma_s > 4.67 \qquad (5b)$$

This equation is based on the considerations of Roels about biomass yields [1983]. The third solution is to approximate the standard Gibbs energy of growth with that of most microorganisms, $\Delta_{met}G^0$ = -500 kJ/C-mol [von Stockar, 2013b]. All three approaches were used in this research.

Gibbs energy of growth was used to find biomass yield, Y, using the equation [von Stockar, 2013b]

$$Y = \frac{\Delta_{cat}G^0}{\Delta_{met}G^0 - \Delta_{bs}G^0} \qquad (6)$$

Standard thermodynamic properties of growth

The biomass yield can be used to combine the catabolic and biosynthesis half-reactions into the full growth reaction [von Stockar, 2013b]. The catabolic and biosynthesis half-reactions are two parts of the total metabolism [von Stockar, 2013b]. They are combined using the biomass yield [von Stockar, 2013b]. The catabolic half-reaction is divided with the biomass yield and added to the biosynthesis half-reaction. Thus, the stoichiometric coefficient of substance i in the total growth reaction, $\nu_{i,met}$, is

$$\nu_{i,met} = \frac{1}{Y}\nu_{i,cat} + \nu_{i,bs} \qquad (7)$$

where $\nu_{i,cat}$ and $\nu_{i,bs}$ are stoichiometric coefficients of substance i in the catabolic and biosynthesis half-reactions, respectively [von Stockar, 2013b].

Similarly, standard thermodynamic parameters of the growth reaction are found by combining those of the catabolic and biosynthesis half-reactions [von Stockar, 2013b]. Standard enthalpy, $\Delta_{met}H^0$, and entropy, $\Delta_{met}S^0$, of growth (metabolism) are given by the equations [von Stockar, 2013b]

$$\Delta_{met}H^0 = \frac{1}{Y}\Delta_{cat}H^0 + \Delta_{bs}H^0 \qquad (8)$$

$$\Delta_{met}S^0 = \frac{1}{Y}\Delta_{cat}S^0 + \Delta_{bs}S^0 \qquad (9)$$

Standard Gibbs energy of growth is calculated using predictive models, as described in the section *Driving force of growth and biomass yield*.

Activities

All thermodynamic parameters discussed thus far are under standard conditions: standard temperature of 298.15 K (25°C) and pressure 1 bar, as well as unit activities of all reactants and products [Atkins and de Paula, 2011, 2014]. However, it would be good to include the influence concentrations and activities of the reactions and products. The influence of activity on reaction Gibbs energy, $\Delta_r G$, is given by the equation

$$\Delta_r G = \Delta_r G^0 + RT \ln Q \tag{10}$$

Where $\Delta_r G^0$ is standard reaction Gibbs energy, R the universal gas constant, T temperature and Q the reaction quotient [Atkins and de Paula, 2011, 2014]. The reaction quotient is defined through the activities and stoichiometric coefficients of the reaction participants [Atkins and de Paula, 2011, 2014].

$$Q = \prod_i a_i^{\nu_i} \tag{11}$$

Where ν_i and a_i are the stoichiometric coefficient and activity of substance i, respectively [Atkins and de Paula, 2011, 2014]. Activity of substance i is given by the equation

$$a_i = \gamma_i \, C_i \tag{12}$$

Where γ_i and C_i are the activity coefficient and molarity of substance i, respectively [Atkins and de Paula, 2011, 2014.

The problem of activities has been approached in 4 ways. The first and easiest method is to set all activities to 1. Substituting this into equation (11) gives $Q = 1$. This means that the $RT \ln Q$ term from equation (10) becomes zero. Thus, the reaction Gibbs energy, $\Delta_r G$, reduces to the standard reaction Gibbs energy, $\Delta_r G^0$. In other words, the influence of activities on reaction Gibbs energy is assumed to be very small and hence negligible. This modifies equations (10-12) into

$$\Delta_r G \approx \Delta_r G^0 \tag{13a}$$

$$Q = 1 \tag{13b}$$

$$a_i = 1 \qquad \text{for all i} \tag{13c}$$

In the present discussion, this means approximating Gibbs energies of biological processes $\Delta_{cat}G$, $\Delta_{bs}G$ and $\Delta_{met}G$ with their standard values $\Delta_{cat}G^0$, $\Delta_{bs}G^0$ and $\Delta_{met}G^0$.

The second approach is to approximate activities of all the components with their concentrations (Table 2). This means that γ_i is set to 1 for all the considered substances in equation (12), leaving $a_i = C_i$ [Atkins and de Paula, 2011, 2014; Skoog et al., 2013]. Substituting this into equation (11) gives the concentration reaction quotient, Q_C [Atkins and de Paula, 2011, 2014; Skoog et al., 2013]. The concentration reaction quotient is then substituted into equation (10) to give reaction Gibbs energy with corrections for concentrations, $\Delta_r G_C$. This means that equations (10-12) become

$$\Delta_r G_C = \Delta_r G^0 + RT \ln Q_C \tag{14a}$$

$$Q_C = \prod_i C_i^{\nu_i} \tag{14b}$$

$$a_i \approx C_i \qquad \text{for all i} \tag{14c}$$

Approximating activities with concentrations means assuming intermolecular forces between molecules in the solution are not very strong and can be neglected [Atkins and de Paula, 2011, 2014; Skoog et al., 2013]. This assumption was used to calculate Gibbs energy of biosynthesis with corrections for concentrations, $\Delta_{bs}G_C$, which will later be compared to the values calculated using the other 3 approaches.

The third approach is to include only the activity coefficients of ions, which interact the strongest, using the extended Debye-Hückel equation. The assumption that there are no intermolecular forces in the solution is the least accurate for ions [Atkins and de Paula, 2011, 2014; Skoog et al., 2013]. Ions are charged and hence interact through coulomb forces, which are strong and have a relatively long range [Atkins and de Paula, 2011, 2014; Skoog et al., 2013]. These interactions are taken into account by the activity coefficients, which can be calculated using the extended Debye-Hückel equation [von Stockar et al., 2013; Maskow and von Stockar, 2005]. According to the extended Debye-Hückel equation, the activity coefficients depend on the ionic force of the solution, I, given by the equation

$$I = \frac{1}{2}\sum_i C_i z_i^2 \qquad (15)$$

Where C_i and z_i are the concentration (molarity) and charge of species i, respectively. The sum is over all the species in the solution [Von Stockar et al., 2013; Maskow and von Stockar, 2005; Atkins and de Paula, 2011, 2014; Skoog et al., 2013]. The ionic force is then used to calculate the activity coefficient of each species, using the extended Debye-Hückel equation

$$log_{10}(\gamma_i) = -\frac{A\, z_i^2\, \sqrt{I}}{1 + B\, a_{ion,i}\, \sqrt{I}} \qquad (16)$$

Where A and B are constants, while $a_{ion,i}$ represents the effective diameter of the hydrated ion i [Von Stockar et al., 2013; Maskow and von Stockar, 2005; Atkins and de Paula, 2011, 2014; Skoog et al., 2013]. For aqueous solutions at 25°C, $A = 0.5085$ and $B = 3.3$ Å^{-1} [Skoog et al., 2013]. Once the values of γ_i are substituted into equation (12), the Debye-Hückel activates are obtained, which are then used in equation (11) to find the Debye-Hückel reaction quotient, Q_{DH}. The Debye-Hückel reaction quotient is substituted into equation (10) to find the reaction Gibbs energy with Debye-Hückel activities, Δ_rG_{DH}. This approach was used to find $\Delta_{bs}G_{DH}$ values.

The fourth and most accurate method is to include activities of uncharged species, such as glucose, in addition to the Debye-Hückel activities for ions. The activity correction for glucose, which is not charged, was calculated as described in [Popovic et al., 2019]. Standard Gibbs energy of formation of glucose *in an aqueous solution* is equal to its standard Gibbs energy of formation *in solid state* plus two corrections: solution and dilution. The correction for solution was taken from [Popovic et al., 2019]. The dilution correction was made using the Margules model, as described in [Popovic et al., 2019]. Once the dilution correction is taken into account, thermodynamic parameters of glucose in an 23.97 μM solution are Δ_fH^0(glc, aq) = -1265.84 kJ/mol, $S_m{}^0$ (glc, aq) = 250.35 J/mol K and Δ_fG^0(glc, aq) = -921.15 kJ/mol. These values are then used to find Δ_rG^{0*}, reaction Gibbs energy with the glucose correction. Δ_rG^{0*} is then added to the reaction quotient calculated using the Debye-Hückel activities, but with the activity of glucose set to 1 (since it was already included into Δ_rG^{0*} no further corrections are needed).

The activity of live matter was set to 1 in all four approaches, since it represents a distinct phase from the surrounding solution. Live matter is present in the solution in the form of cells (E. coli) or virus particles (T4 and Lambda phages). Cells are separated from the growth medium by the cell membrane and hence represent a distinct phase. Similarly, T4 and Lambda phages possess protein capsids, which separate the virus particles from the growth medium and make them a distinct phase. Thus, since both cells and virus particles represent separate phases from the growth medium, their activities are $a_{bio} = 1$ [Atkins and de Paula, 2011, 2014]. Similarly, water is the solvent and its activity is $a_{H2O} = 1$ [Atkins and de Paula, 2011, 2014].

Results

Equations for calculating stoichiometric coefficients for biosynthesis reactions on the M9 growth medium were formulated. They are given in Table 4. Elemental composition of live matter was used to formulate equations for stoichiometric coefficients for microorganism biosynthesis on the M9 medium. The equations from Table 4 were combined with the elemental compositions of live matter from Table 1 to find the values of the stoichiometric coefficients of the analyzed organisms, given in Table 5. The stoichiometric coefficients from Table 5 are for the general growth reaction on the M9 growth medium

$$C_6H_{12}O_6 + NH_4^+ + HPO_4^{2-} + SO_4^{2-} + O_2 \rightarrow (Bio) + H_2PO_4^- + H_2O \qquad (17)$$

where (Bio) denotes live mater with elemental composition from Table 1.

Table 5: Values of the stoichiometric coefficients for the biosynthesis half-reactions of the analyzed organisms. The values were calculated using the formulas from Table 3 and elemental composition of live matter from Table 1. The values of stoichiometric coefficients of products are positive, while those of reactants are negative.

Organism	$C_6H_{12}O_6$ (aq)	NH_4^+ (aq)	HPO_4^{2-} (aq)	SO_4^{2-} (aq)	O_2 (g)	Bio	$H_2PO_4^-$ (aq)	H_2O (l)
T4 phage	-0.1667	-0.3120	-0.26584	-0.0032	-0.0267	1	0.2260	0.8085
Lambda phage	-0.1667	-0.3271	-0.26989	-0.0031	-0.0460	1	0.2188	0.8618
E. coli	-0.1667	-0.24	-0.24	0	0.0175	1	0.24	0.475
E. coli	-0.1667	-0.26	-0.26	0	0.0080	1	0.26	0.52

Stoichiometric coefficients from Table 5 were combined with standard thermodynamic properties of live matter from Table 3, to find standard thermodynamic properties of biosynthesis, using the Hess's law (equations 1-3). Standard thermodynamic properties of biosynthesis include standard enthalpy of biosynthesis, $\Delta_{bs}H^0$, standard entropy of biosynthesis, $\Delta_{bs}S^0$, and standard Gibbs energy of biosynthesis, $\Delta_{bs}G^0$. Standard thermodynamic properties of biosynthesis of the T4 phage, Lambda phage and E. coli live matter are given in Table 6. Standard enthalpy of biosynthesis for E. coli is between -6.9 and -10.4 kJ/C-mol, while those of the T4 and Lambda phages are -18.6 kJ/C-mol and -25.4 kJ/C-mol, respectively. Standard entropy of biosynthesis of E. coli is between 35.3 and 35.8 J/C-mol K, while those of the T4 and Lambda phages are 35.8 and 33.6 kJ/C-mol, respectively. Standard Gibbs energy of biosynthesis of E. coli is between -17.6 and -21.4 kJ/C-mol, while those of the T4 and Lambda phages are -29.9 kJ/C-mol and -36.1 kJ/C-mol, respectively.

Table 6: Standard thermodynamic properties of biosynthesis of the analyzed organisms. $\Delta_{bs}H^0$ denotes standard enthalpy of biosynthesis. $\Delta_{bs}S^0$ denotes standard entropy of biosynthesis. $\Delta_{bs}G^0$ denotes standard Gibbs energy of biosynthesis. The values in this table were calculated using the Hess's law, combining the stoichiometric coefficients from Table 5 and standard thermodynamic properties of live matter from Table 3. .

Organism	$\Delta_{bs}H^0$ (kJ/C-mol)	$\Delta_{bs}S^0$ (J/C-mol K)	$\Delta_{bs}G^0$ (kJ/C-mol)
T4 phage	-18.6	35.8	-29.9
Lambda phage	-25.4	33.6	-36.1
E. coli	-6.9	35.3	-17.6
E. coli	-10.4	35.8	-21.4

Standard Gibbs energies of biosynthesis were combined with the medium composition, to find corrections for activities of the reactants and products. The results are given in Table 7. The composition of the growth medium is given in Table 2. The activities were treated in four ways, as described in the section Activities. The first and simplest method is assuming that activities of all the substances are equal to 1. This means that they give a negligible contribution to the Gibbs energy of biosynthesis in equation (10), giving equation (13) and hence $\Delta_{bs}G \approx \Delta_{bs}G^0$. The second approach is to approximate activities with concentrations of substances, using equation (14). This results in the concentration reaction quotient,

Q_c, and Gibbs energy of biosynthesis with concentrations taken into account, $\Delta_{bs}G_c$, in Table 7. The third approach is to include activity coefficients of ions only, using the extended Debye-Hückel equation (16). The Debye-Hückel activity coefficients are substituted into equations (10-12). The result is the Debye-Hückel reaction quotient, Q_{DH}, and Debye-Hückel Gibbs energy of biosynthesis, $\Delta_{bs}G_{DH}$, values in Table 7. The fourth approach is to include activity of glucose, in addition to that of ions, using the approach described in [Popovic et al., 2019]. This results in the Q_a and $\Delta_{bs}G$ values in Table 7.

Table 7: Comparison of values of Gibbs energies of biosynthesis obtained with various activity corrections. $\Delta_{bs}G^0$ is the standard Gibbs energy of biosynthesis (with activities of all substances set to 1). $\Delta_{bs}G_C$ is Gibbs energy of biosynthesis calculated using concentrations of all the substances (Table 2), but without activity coefficients (which were set to 1). Q_C represents the reaction quotient calculated using concentrations. $\Delta_{bs}G_{DH}$ is Gibbs energy of biosynthesis calculated with activity coefficients obtained using the extended Debye-Hückel equation [Von Stockar et al., 2013; Maskow and von Stockar, 2005], while the activity coefficients of uncharged species were set to 1. Q_{DH} represents the reaction quotient calculated using the extended Debye-Hückel equation. $\Delta_{bs}G$ is Gibbs energy of biosynthesis with all the activity coefficients included, including that of glucose [Popovic et al., 2019]. Q_a represents the reaction quotient calculated using activities of all the reactants and products.

Organism	$\Delta_{bs}G^0$ (kJ/C-mol)	ln(Q_c)	$\Delta_{bs}G_c$ (kJ/C-mol)	ln(Q_{DH})	$\Delta_{bs}G_{DH}$ (kJ/C-mol)	ln(Q_a)	$\Delta_{bs}G$ (kJ/C-mol)
T4 phage	-29.9	3.512	-21.2	3.866	-20.3	2.093	-24.07
Lambda phage	-36.1	3.783	-26.7	4.149	-25.8	2.376	-29.54
E. coli	-17.6	2.647	-11.0	2.939	-10.3	1.166	-14.04
E. coli	-21.4	2.811	-14.5	3.127	-13.7	1.354	-17.41

All the analyzed organisms are growing aerobically on the M9 medium with glucose. Thus, their carbon and energy source is glucose, while the electron acceptor is oxygen. Therefore, the catabolic half-reaction is oxidation of glucose by O_2. The catabolic half-reaction and its thermodynamic properties are given in Table 8. For every mole of glucose consumed, $\Delta_{cat}H^0$ = -2802.74 kJ/mol of heat is released. Standard entropy of catabolism is 258.97 J/mol K. Thus, the catabolic half-reaction has a highly negative standard Gibbs energy change of -2871.74 kJ/mol. This value can be more accurate by including the correction for activities, as described in the *Activities* section. This gives the Gibbs energy of the catabolic half-reaction of -2861.93 kJ/mol.

Table 8: Stoichiometry and thermodynamic parameters of the catabolic half-reaction. For all the analyzed organisms, the catabolic half-reactions are identical. The stoichiometric coefficients of the products are positive, while those of the reactants are negative. $\Delta_{cat}H^o$, $\Delta_{cat}S^o$ and $\Delta_{cat}G^o$ denote standard enthalpy, entropy and Gibbs energy of catabolism, respectively. Q_a represents the reaction quotient calculated using the activities of the products and reactants. The activity of glucose was set to one, because its thermodynamic properties were calculated as described in the methods section. $\Delta_{cat}G$ denotes Gibbs energy of catabolism with included corrections for activities of the products and reactants.

Stoichiometry				
$C_6H_{12}O_6$ (aq)	O_2 (g)	CO_2 (aq)	H_2O (l)	
-1	-6	6	6	
Thermodynamic parameters				
$\Delta_{cat}H^o$ (kJ/mol)	$\Delta_{cat}S^o$ (J/mol K)	$\Delta_{cat}G^o$ (kJ/mol)	ln(Q_a)	$\Delta_{cat}G$ (kJ/mol)
-2802.74	258.97	-2871.74	-17.92	-2861.93

Catabolic and biosynthesis half-reactions are combined to find the total growth reaction [von Stockar, 2013b]. Gibbs energy of growth (metabolism), $\Delta_{met}G^o$, was calculated using equation (4) by Heijnen and van Dijken [1992], equation (5) by Liu et al. [2007], and the approximate value for most microorganisms: -500 kJ/C-mol [von Stockar, 2013b]. The results are given in Table 9. These were combined with standard Gibbs energies of catabolic and biosynthetic half-reactions to find the biomass yield, Y, using equation (6).

Table 9: Substrate degree of reduction, standard Gibbs energy of metabolism and biomass yields of the analyzed organisms. The substrate for all the analyzed organisms is glucose with a degree of reduction $\Gamma_s = 4$. $\Delta_{met}G^0$ denotes standard Gibbs energy of metabolism, including both catabolism and anabolism. $\Delta_{met}G^0$ was calculated using three models: Heijnen equation [Heijnen and van Dijken, 1992], Liu equation [Liu et al., 2007] and approximate value for most microorganisms [von Stockar, 2013b]. $\Delta_{cat}G^0$ and $\Delta_{bs}G^0$ represent standard Gibbs energies of catabolism and biosynthesis, respectively. Y denotes the biomass yield, calculated using the equation $Y = \Delta_{cat}G^0 / (\Delta_{met}G^0 - \Delta_{bs}G^0)$ [von Stockar, 2013b]. Y was calculated for all three methods used to find $\Delta_{met}G^0$.

Organism	Substrate	Γ_S	$\Delta_{met}G^0$ (kJ/C-mol)			$\Delta_{cat}G^0$ (kJ/C-mol)	$\Delta_{bs}G^0$ (kJ/C-mol)	Y		
			Heijnen	Liu	Approx			Heijnen	Liu	Approx
T4 phage	**$C_6H_{12}O_6$**	**4.0**	**-236.1**	-409.7	-500	-2871.7	-29.9	13.9	7.6	6.1
Lambda phage	$C_6H_{12}O_6$	4.0	-236.1	-409.7	-500	-2871.7	-36.1	14.4	7.7	6.2
E. coli	$C_6H_{12}O_6$	4.0	-236.1	-409.7	-500	-2871.7	-17.6	13.1	7.3	6.0
E. coli	$C_6H_{12}O_6$	4.0	-236.1	-409.7	-500	-2871.7	-21.4	13.4	7.4	6.0

The biomass yield was used to combine the catabolic and biosynthetic half-reactions to find the total growth reaction, using equation (7). The stoichiometric coefficients for the total growth reactions of the analyzed organisms are given in Table 10. Moreover, the biomass yields were used to find standard enthalpy, $\Delta_{met}H^0$, and entropy, $\Delta_{met}S^0$, of growth (metabolism), using equations (8) and (9). Their values are shown in Table 11. Finally, the correction for activities was made using equations (10-12), giving Gibbs energy of growth (metabolism), $\Delta_{met}G$. The values of $\Delta_{met}G$ for the analyzed organisms, along with the Gibbs energies of catabolism and biosynthesis, are shown in Table 12.

Table 10: Stoichiometric coefficients of entire growth reactions, including both catabolism and anabolism, of the analyzed organisms. Entire growth reactions were obtained by combining the catabolic and anabolic half-reactions, using the biomass yield Y, as described in the Methods section. The stoichiometric coefficient of substance i in the entire growth reaction, $\nu_{i,met}$, is given as $\nu_{i,met} = (1/Y)\ \nu_{i,cat} + \nu_{i,bs}$, where Y is the biomass yield (Table 9), while $\nu_{i,cat}$ and $\nu_{i,bs}$ denote the stoichiometric coefficient of that substance in the catabolic (Table 4) and biosynthesis (Table 8) half-reaction, respectively. The stoichiometric coefficients of the products are positive, while those of the reactants are negative.

Organism	$C_6H_{12}O_6$ (aq)	NH_4^+ (aq)	HPO_4^{2-} (aq)	SO_4^{2-} (aq)	O_2 (g)	Bio	$H_2PO_4^-$ (aq)	H_2O (l)	CO_2 (aq)
T4 phage	-0.2989	-0.3120	-0.2658	-0.0032	-0.8201	1	0.2260	1.6019	0.7934
Lambda phage	-0.2967	-0.3271	-0.2699	-0.0031	-0.8265	1	0.2188	1.6422	0.7805
E. coli	-0.3032	-0.2400	-0.2400	0.0000	-0.8016	1	0.2400	1.2941	0.8191
E. coli	-0.3019	-0.2600	-0.2600	0.0000	-0.8031	1	0.2600	1.3311	0.8111

Table 11: Standard thermodynamic properties of entire metabolic reactions of the analyzed organisms. $\Delta_{met}H^0$, $\Delta_{met}S^0$ and $\Delta_{met}G^0$ denote standard enthalpy, entropy and Gibbs energy of metabolism (both catabolism and biosynthesis included). They were calculated using the equation $\Delta_{met}X^0 = (1/Y)\ \Delta_{cat}X^0 + \Delta_{bs}X^0$, where X denotes a thermodynamic property (H or S), Y the biomass yield (Table 9), while $\Delta_{cat}X^0$ and $\Delta_{bs}X^0$ denote the corresponding thermodynamic property of the catabolic and biosynthetic half-reactions, respectively.

Organism	$\Delta_{met}H^0$ (kJ/C-mol)	$\Delta_{met}S^0$ (J/C-mol K)	$\Delta_{met}G^0$ (kJ/C-mol)
T4 phage	-389.2	70.0	-409.7
Lambda phage	-390.0	67.3	-409.7
E. coli	-389.5	70.6	-409.7
E. coli	-389.3	70.8	-409.7

Table 12: Gibbs energies of catabolism, biosynthesis and entire metabolism of the analyzed microorganisms, including the activities of the nutrients and metabolic products. $\Delta_{cat}G$, $\Delta_{bs}G$ and $\Delta_{met}G$ represent Gibbs energies of catabolism, biosynthesis and metabolism, respectively.

Organism	$\Delta_{cat}G$ (kJ/C-mol)	$\Delta_{bs}G$ (kJ/C-mol)	$\Delta_{met}G$ (kJ/C-mol)
T4 phage	-2861.93	-24.07	-402.5

Lambda phage	-2861.93	-29.54	-401.8
E. coli	-2861.93	-14.04	-404.8
E. coli	-2861.93	-17.41	-404.3

Discussion

This paper analyzes the interaction of E. coli with T4 and Lambda bacteriophages, on the M9 growth medium with glucose. Thus, elemental compositions and standard thermodynamic properties of the analyzed microorganisms were taken from the literature [Bauer and Ziv, 1976; Battley, 1992; Popovic, 2019; Popovic and Minceva, 2020a]. The composition of the growth medium was also taken from the literature [Geerlof, 2010; Cold Spring Harbor Protocols, 2022; Xing et al., 2014], as well as the thermodynamic properties of its constituents [Atkins and de Paula, 2011; 2014; Rard and Wolery, 2007; Kielland, 1937; Skoog et al., 2013]. These were combined to find the catabolic and biosynthetic half-reactions for E. coli, and T4 and Lambda phages. Moreover, standard thermodynamic properties of catabolism and biosynthesis were calculated, as well as thermodynamic properties with corrections for activities of substances in the growth medium. Finally, the driving force of growth and complete growth reactions were formulated for the analyzed microorganisms.

Biosynthesis

Table 5 gives the stoichiometric coefficients for the biosynthesis half-reactions for the analyzed microorganisms. The stoichiometric values for O_2 are different for the bacteriophages and the host cells. For the bacteriophages they are negative, meaning oxygen is consumed for biosynthesis, in excess to that used in catabolism [von Stockar, 2013b; Heijnen, 2013]. On the other hand, for E. coli they are positive, meaning that no additional oxygen is used for biosynthesis [von Stockar, 2013b; Heijnen, 2013]. The reason for this is the higher nitrogen content of viruses. The consumption of O_2 in biosynthesis of T4 and Lambda phages means that the substrate is partly oxidized while it is incorporated into phage live matter. The oxidation process is exothermic. Thus, the two bacteriophages have more negative standard enthalpies of biosynthesis (Table 6). T4 phage has a standard enthalpy of biosynthesis of -18.6 kJ/C-mol, for Lambda phage it is -25.4 kJ/C-mol, while for E. coli $\Delta_{bs}H^0$ is between -6.9 and -10.4 kJ/C-mol. Moreover, the more negative enthalpies make standard Gibbs energies of biosynthesis of the T4 and Lambda phages more negative that of their host E. coli, according to the equation $\Delta_{bs}G^0 = \Delta_{bs}H^0 - T\,\Delta_{bs}S^0$. Thus, for the T4 phage standard Gibbs energy of biosynthesis is -29.9 kJ/C-mol, for the Lambda phage it is -36.1 kJ/C-mol, while for E. coli $\Delta_{bs}G^0$ is between -17.6 and -21.4 kJ/C-mol (Table 6). Therefore, the chemical structure of phages is reflected in their elemental composition. The elemental composition of makes standard Gibbs energy of biosynthesis of phages different from that of their host cell. The more negative Gibbs energy of biosynthesis allows viruses to hijack their host cell metabolism [Popovic, 2022i]. Thus, biothermodynamic analysis reveals how the chemical structure of phages ultimately allows them to multiply inside their host cells, due to more negative Gibbs energy of biosynthesis.

Standard thermodynamic properties of biosynthesis of the T4 phage, Lambda phage and E. coli live matter are given in Table 6. For all three organisms, standard enthalpies of biosynthesis are slightly negative, meaning that the biosynthesis process is slightly exothermic. Standard entropies of biosynthesis are positive, due to the increase in the total number of particles during the process. On the product side of the biosynthesis reactions, the total number of particles is between 1.7 and 2, while on the reactant side it is between 0.6 and 0.8 (Table 5). The large number of particles on the product side is due to the released water molecules and synthetized biomass. Thus, the total number of particles increases during the process, leading to positive entropy change. On the other hand, all standard Gibbs energies of biosynthesis are slightly negative, between -17.6 and -36.1 kJ/C-mol (Table 6). This means that the biosynthesis process itself is a spontaneous process on the M9 medium. However, the standard Gibbs energies of biosynthesis are only slightly negative, meaning that the process would be very slow, which is inadequate for living organisms [von Stockar, 2013b]. Thus, additional negative Gibbs energy must be made available by the catabolism to make the process proceed at the required rate [von Stockar, 2013b].

Table 6 also shows that standard Gibbs energies of biosynthesis of the T4 and Lambda bacteriophages are between 1.5 and 2 times more negative than those of their host E. coli. The more negative Gibbs energy of biosynthesis allows the viruses to hijack the host cell metabolism [Popovic and Minceva, 2020a, 2020b]. The negative Gibbs energy of biosynthesis represents the thermodynamic driving force for biosynthesis for both bacteria [von Stockar, 2013a, 2013b] and viruses [Popovic and Minceva, 2020a, 2020b]. Gibbs energy of biosynthesis is proportional to the biosynthesis rate – the rate of formation of new live matter [Demirel, 2014]. Thus, due to their more negative Gibbs energy of biosynthesis, the T4 and Lambda phages are able to hijack the host cell metabolism.

Standard Gibbs energies of biosynthesis are defined under standard conditions: standard temperature of 298.15 K (25°C) and pressure 1 bar, as well as unit activities of all reactants and products [Atkins and de Paula, 2011, 2014]. Thus, using standard Gibbs energies of biosynthesis implies assuming that activities of all substances in the biosynthesis reaction do not greatly influence Gibbs energy of biosynthesis [von Stockar, 2013b; von Stockar et al., 2013; Heijnen, 2013]. This is true in case of large absolute values of Gibbs energy [von Stockar, 2013b; von Stockar et al., 2013; Heijnen, 2013]. However, $\Delta_{bs}G^0$ for the analyzed organisms on the M9 medium is not highly negative. Thus, it is interesting to see the influence of activities, which are given in Table 7.

Table 7 gives standard Gibbs energies of biosynthesis with different corrections for activities of reactants and products. Approximating $\Delta_{bs}G$ with its standard value $\Delta_{bs}G^0$ is the least accurate method. A more accurate approach is to approximate activities of substances with their concentrations, giving the $\Delta_{bs}G_c$ values. Even more accurate is to include the activity coefficients of ions, which interact the strongest, using the extended Debye-Hückel equation. This results in the $\Delta_{bs}G_{DH}$ values. Finally, the most accurate solution is to include the activity of uncharged species, like glucose, giving the $\Delta_{bs}G$ values. The data in Table 7 reveals that, regardless of the method used, the ratio of Gibbs energies of T4 and Lambda phages to that of their host cells remains similar. The bacteriophages always have a more negative Gibbs energy of biosynthesis than their host Escherichia coli. However, since the absolute values of $\Delta_{bs}G$ are relatively small for all the analyzed organisms, it is good to include the activity corrections. For example, $\Delta_{bs}G^0$ of the Lambda phage is -36.1 kJ/C-mol, while after the activity correction, it becomes $\Delta_{bs}G$ = -29.54 kJ/C-mol. The activity corrections for the analyzed microorganisms are between 22 and 25% of the final corrected $\Delta_{bs}G$ value.

Catabolism

The Gibbs energies of biosynthesis of the analyzed microorganisms do not provide a sufficient driving force for their metabolism to proceed at required rates [von Stockar, 2013a, 2013b; Hellingwerf et al., 1982; Westerhoff et al., 1982]. Thus, additional driving force must be provided by the catabolism [von Stockar, 2013a, 2013b; Hellingwerf et al., 1982; Westerhoff et al., 1982]. The catabolism degrades the substrate molecules into simple products, releasing free energy to drive the metabolism [von Stockar, 2013a, 2013b; Berg et al., 2002]. Catabolism of the analyzed organisms was described using a catabolic-half reaction. Since all the analyzed organisms are growing on the same medium, their catabolic half-reactions are identical. The stoichiometry and thermodynamic properties of the catabolic half-reactions are given in Table 8. The stoichiometry of the catabolic half-reaction is that of aerobic glucose catabolism. Standard enthalpy of catabolism $\Delta_{cat}H^0$ = -2802.74 kJ/C-mol is much more negative than that of biosynthesis, meaning that catabolism is highly exothermic. The highly negative $\Delta_{cat}H^0$ results in a high thermodynamic driving force for the catabolic half-reaction: standard Gibbs energy of catabolism is $\Delta_{cat}G^0$ = -2871.74 kJ/C-mol. Due to the large absolute value of $\Delta_{cat}G^0$, the activity correction is only minor, changing it to $\Delta_{cat}G$ = -2861.93 kJ/C-mol. The highly negative Gibbs energy of catabolism provides the thermodynamic driving force that allows both bacteriophages and their host cells to multiply at required rates.

Driving force of growth and biomass yield

Table 9 shows standard Gibbs energies of growth (metabolism), $\Delta_{met}G^0$, and biomass yields, Y, for the analyzed organisms. Standard Gibbs energy of growth depends on the degree of reduction of and the number of carbon atoms in the substrate [Heijnen, 2013; Heijnen and van Dijken, 1992]. Since all the analyzed microorganisms are growing on the same M9 medium with glucose, the substrate is the same for all – glucose. Glucose, $C_6H_{12}O_6$, has a degree of reduction of 4 and contains 6 carbon atoms. Substituting these values into equations (4) and (5) gives the driving forces of growth, $\Delta_{met}G^0$, for the analyzed microorganisms, which are shown in Table 9. For all the analyzed organisms, $\Delta_{met}G^0$ are the same, since all are using the same substrate. The $\Delta_{met}G^0$ values were calculated using three methods: equation (4) by Heijnen and van Dijken [1992], equation (5) by Liu et al. [2007] and the average value for most microorganisms [von Stockar, 2013b].

The $\Delta_{met}G^0$ value given equation (5) is -409.7 kJ/C-mol, which is similar to the value for most microorganisms, -500 kJ/C-mol. On the other hand, the value given by equation (4), -236.1 kJ/C-mol, is less negative. Both the bacteriophages and E. coli have identical $\Delta_{met}G^0$ values, since $\Delta_{met}G^0$ depends only on the substrate, which is identical for both.

Even though, the $\Delta_{met}G^0$ is identical for the phages and bacteria, standard Gibbs energies of biosynthesis are significantly different, $\Delta_{bs}G^0$. Thus, it seems that $\Delta_{bs}G^0$ decides which organism will win the competition for the metabolic machinery. The organism that controls the metabolic machinery is better adapted to the environment and has an advantage. Even though the difference in Gibbs energies of biosynthesis is double, one should have in mind that the values of $\Delta_{bs}G^0$ themselves are not very great. Thus, numerically, this advantage is such that it enables the multiplication of the bacteriophages, but does not endanger the survival of the bacteria, enabling both species to survive the competitive conditions.

Bacteria and phages are known to coexist in many ecosystems, where they interact [De Paepe and Petit, 2014; Jover et al., 2014]. A dynamic equilibrium exists between the bacteria and phages [De Paepe and Petit, 2014; Jover et al., 2014]. Thus, even though phages infect bacteria, they are not able to completely dominate the ecosystem and make the competition process irreversible. In the ecosystem, both the bacteria and the phages coexist. This observation can be explained by the small difference in Gibbs energies of biosynthesis of the bacteria and phages. On the other hand, Gibbs energies of metabolism are similar and explain the existence of both kinds of microorganisms. Figure 1 shows Gibbs energies of biosynthesis and metabolism of the analyzed organisms. From Figure 1 we see that Gibbs energies of metabolism are similar for all the analyzed organisms. On the other hand, Gibbs energies of biosynthesis of the phages two times more negative than that of E. coli.

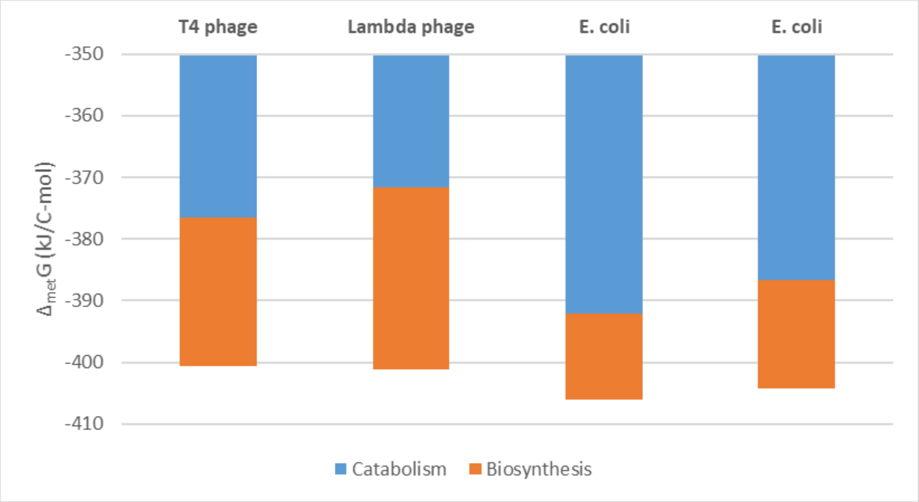

Figure 1: Gibbs energies of catabolism, biosynthesis and metabolism of T4 and Lambda phages, and their host E. coli. The contributions of Gibbs energies of catabolism ($\Delta_{cat}G/Y$, blue) and biosynthesis ($\Delta_{bs}G$, orange) to the total Gibbs energy of metabolism. Gibbs energies of metabolism are very similar for all the analyzed organisms. However, the values of $\Delta_{bs}G$ of the phages are two times larger than that of E. coli. Note: for easier presentation, the graph begins at -350, not at 0.

Entire metabolism

Gibbs energies of metabolism were used to find biomass yields (Table 9). The biomass yields were found for all three models. The values given by the model by Liu et al. [2007] were chosen for further analysis, since the model has a better theoretical foundation and gives results more similar to that of most microorganisms (-500 kJ/C-mol) [von Stockar, 2013b]. Biomass yields were used to find stoichiometric coefficients for the entire metabolism, which are presented in Table 10, as well as standard enthalpy and entropy of metabolism (growth), given in Table 11. The data from Tables 10 and 11 were combined to find the influence of activities on Gibbs energy of metabolism, given in Table 12.

Gibbs energy of metabolism has a relatively large value for the analyzed microorganisms, between -402 and -405 kJ/C-mol. Thus, the influence of activities of nutrients and metabolic products is relatively small. This is in accordance with the predictions of von Stockar et al. [2013]. Therefore, it is good to take activities into account when discussing processes with a relatively low driving force, such as biosynthesis on the M9 medium. However, activities are less important when considering processes with a greater driving force, like entire metabolism and catabolism.

Conclusions

The driving forces for metabolism of bacteriophages and bacteria are identical - Gibbs energy. The driving force is shared between different classes of organisms, since they all evolved from the same last universal common ancestor (LUCA). The only differences is that the organisms took different pathways through evolution: bacteria evolved towards greater complexity, while phages evolved towards simplicity. However, bacteria and phages differ in the magnitude of Gibbs energy of biosynthesis. The phages have a more negative Gibbs energy of biosynthesis than bacteria, enabling them to hijack their metabolism. The absolute difference between Gibbs energies of biosynthesis of phages and bacteria is not very great. This allows both phages and bacteria to coexist in ecosystems.

Since the magnitudes of Gibbs energies of biosynthesis on M9 medium are not very great, the activities of nutrients and metabolic products change their values by between 22 and 25%. Thus, it is good to take activities into account when discussing biosynthesis on the M9 medium. On the other hand, Gibbs energies of catabolism and metabolism as a whole are much greater and activities make only a minor correction.

Compliance with Ethical Standards

The author declares no conflict of interest.
No animals were used in the research.
No human participants took part in the research.

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